NATURE

No. 4798 SATURDAY, OCTOBER 14, 1961 Vol. 192

CONTENTS

	Page
THE AMATEUR IN SCIENCE	95
MATHEMATICAL TABLES. By Prof. C. A. Coulson, F.R.S.	97
ORIGIN OF THE NORTHERN RHODESIAN COPPER DEPOSITS. By Prof. J. H. Taylor, F.R.S.	98
SCIENCE IN POETRY AND VERSE. By T. R. Henn, C.B.E.	98
A NEW LOOK IN PHYSICS TEXT-BOOKS. By G. R. Noakes	99
MATERIALS FOR REACTORS. By B. W. Mott	100
ENZYMES AND CANCER. By Dr. L. H. Stickland	100
PHYSICAL OCEANOGRAPHY. By Dr. J. C. Swallow	101
ARSENIC CONTENT OF NAPOLEON I'S HAIR PROBABLY TAKEN IMMEDIATELY AFTER HIS DEATH. By Dr. Sten Forshufvud, Dr. Hamilton Smith and Dr. Anders Wassén	103
TEACHING MACHINES. By Prof. H. Kay	105
BREATHING. By Flight-Lieut. D. H. Glaister	106
THE NORMAN LOCKYER OBSERVATORY. By Prof. G. K. T. Conn	108
LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES	109
THE SWEDISH SEED ASSOCIATION. By F. R. Horne, C.B.E.	110
OBITUARY: PROF. ERIC BRADSHAW, M.B.E. By Dr. C. Adamson	111
NEWS AND VIEWS	112
COAL SCIENCE. By Dr. I. G. C. Dryden	116
BIOCHEMICAL ENGINEERING. By Dr. D. J. D. Hockenhull	119
COSMIC RAY RESEARCH IN LATIN AMERICA. By Dr. H. Elliot	121
PHARMACEUTICAL CONTROL IN BRITAIN	122
THE LABORATORY OF THE GOVERNMENT CHEMIST. By Dr. William I. Stephen	122
AMSTERDAM CONFERENCE ON PARTICLE ACCELERATORS	123
CONTINENTAL AND OCEANIC DIFFERENTIATION. By Prof. J. D. Bernal, F.R.S.; Dr. Robert S. Dietz; Prof. J. Tuzo Wilson	123
CARBONYL CONTENT OF THE VITRAIN COMPONENT OF BITUMINOUS COALS. By Dr. K. Littlewood and M. T. Monaghan	128
FORMATION OF CRACKS IN ICE PLATES BY THERMAL SHOCK. By L. W. Gold	130
PHOTOCHEMICAL PROPERTIES OF WATER-SOLUBLE CHLOROPHYLL. By Prof. Gerald Oster and Suse B. Broyde	132
A QUANTITATIVE THEORY OF THE CHARACTER OF CHEMICAL BONDING IN BINARY COMPOUNDS. By Prof. Edward Görlich	133
CHITINASE: AN ADDITION TO THE LIST OF HYDROLASES IN THE DIGESTIVE TRACT OF VERTEBRATES. By Dr. Charles Jeuniaux	135
A PRECIPITATING ANTIBODY TO INSULIN. By Valerie E. Jones and Prof. A. C. Cunliffe	136
ANTIBIOTIC PROPERTIES OF MYCELIUM AND METABOLITES OF *Myrothecium roridum* TODE. By Andrzej Nespiak, Marian Kocór and Antoni Siewiński	138
EFFECT OF WHOLE-BODY IRRADIATION AND CORTISONE ON THE DEVELOPMENT OF *Ostertagia* SPP. IN SHEEP. By J. D. Dunsmore	139
HEART DIPOLE REGIONS AND THE MEASUREMENT OF DIPOLE MOMENT. By Dr. M. R. Barber and Dr. E. J. Fischmann	141
CATION-EXCHANGE CAPACITIES OF TISSUES OF HIGHER AND LOWER PLANTS AND THEIR RELATED URONIC ACID CONTENTS. By A. H. Knight, W. M. Crooke and R. H. E. Inkson	142
IMPORTANCE OF OSMOTIC POTENTIAL AS A COMPONENT OF THE TOTAL POTENTIAL OF THE SOIL WATER ON THE MOVEMENT OF NEMATODES. By C. D. Blake	144
CHROMOSOMAL ABERRATIONS INDUCED BY AN ANIMAL VIRUS. By Dr. Berge Hampar and Dr. Solon A. Ellison	145
INFLUENCE OF THE AGE OF POLLEN AND STIGMAS ON SEX DETERMINATION IN HEMP. By Regina Laskowska	147
SIMULATED WAVE-RIDING DOLPHINS. By Byrne Perry, A. J. Acosta and Taras Kiceniuk	148

(Continued overleaf)

a passage to Holland . . .

. . . or it could well have been Basle, Capetown, Manchester, Melbourne, Uppsala or Trail B.C.

In Universities and Industrial Research Laboratories the world over the SP100 Infrared Vacuum Spectrophotometer is providing a combination of high resolution, extreme quantitative accuracy, speed and reliability otherwise unobtainable in one instrument.

Write for the comprehensive brochure giving full details of this remarkable, versatile and powerful spectroscopic tool.

UNICAM

SP 100

Infrared Spectrophotometer

is the complete answer to the most exacting demands of present day analysis

UNICAM INSTRUMENTS LIMITED · YORK STREET · CAMBRIDGE · ENGLAND

LETTERS TO THE EDITORS

Page

SPACE SCIENCE

Errors in Orbital Predictions for Artificial Satellites of Earth.—Kenneth Moe 151

ASTROPHYSICS

Secular Variations of Short-lived Sunspots.—T. S. Ringnes 151

RADIOPHYSICS

Coincidence of the Explosive Phase of Solar Flares with 10·7-cm. Solar Noise Bursts.—Arthur E. Covington and Gladys A. Harvey 152

OCEANOGRAPHY

Sea Surface Temperature in Coastal Waters of the British Isles.—F. E. Lumb 153

PHYSICS

X-Ray and Neutron Diffraction Examination of *p*-Diphenylbenzene.—Prof. C. J. B. Clews, Dr. E. N. Maslen, H. M. Rietveld and T. M. Sabine . . 154

Energy-Level Model for High-Resistivity Gallium Arsenide.—J. Blanc and L. R. Weisberg . . 155

GEOLOGY

Natural Glass from beneath Red Clay on the Floor of the Atlantic.—Dr. G. D. Nicholls and V. T. Bowen . 156

Ultrabasic Liquids.—Dr. H. I. Drever, R. Johnston and C. M. Thomas 157

METALLURGY

Fatigue under Random Loads.—Dr. A. N. May . 158

CHEMISTRY

Formation of Active Nitrogen using Sources of Alternating Current and Direct Current.—G. G. Mannella . 159

Microwave Spectrum of Fluoro-Acetonitrile.—B. E. Job and Dr. J. Sheridan 160

Rubeanic Acid as Stabilizer of Aqueous Solutions of Ascorbic Acid.—Aleksandra Smoczkiewiczowa and Janina Grochmalicka 161

Ligand Field for Chromic Ions in the Corundum Lattice.—Dr. J. Graham and Dr. D. E. Scaife . . . 161

BIOCHEMISTRY

Biosynthesis of L-Ascorbic Acid: Missing Steps in Animals incapable of synthesizing the Vitamin.—I. B. Chatterjee, N. C. Kar, N. C. Ghosh and Prof. B. C. Guha 163

Riboflavin and Liver Sulphydryl-Levels in Mice.—Dr. G. Calcutt, D. Doxey and Joan Coates . . 164

In vivo and *in vitro* Inhibition of Aromatic Hydroxylation by Riboflavin.—K. H. Harper and Dr. G. Calcutt 165

Effect of Insulin on the Phosphorus-32 Mononucleotide Labelling of the Isolated Rat Diaphragm in the Controlled Absence of Extracellular Glucose.—P. Volfin, D. Eboué and H. Clauser 166

Effects of the Sulphur Amino-Acids on the Vitamin A Content in Liver of Rats.—Tatsuo Koyanagi and Satoshi Odagiri 168

Conservation of the Biological Activity of a Soluble Ribonucleic Acid.—J. H. Weil, Prof. J. P. Ebel and R. Monier 169

Mechanism of the Hepatoxic Action of Dialkylnitrosamines.—Dr. D. F. Heath 170

PHYSIOLOGY

Cytotoxic Effect of Aminoacetonitrile on Fibroblast Cultures and its Prevention by Histidine.—Dr. J. Mager, Z. Fuchs, S. Halevy and I. Sciaky . . 170

Incorporation of ^{14}C-glycine by Sub-Nuclear Preparations from Rat Liver Nuclei.—K. R. Rees, Hazel F. Ross and G. F. Rowland 171

Page

Fate of Tritiated Noradrenaline at the Sympathetic Nerve-Endings.—G. Hertting and J. Axelrod . . 172

Phenylalanine as Inhibitor of Transport of Amino-Acids in Brain.—K. D. Neame 173

Stability of Dextran during Prolonged Storage.—Dr. W. d'A. Maycock and Dr. C. R. Ricketts . . 174

PHARMACOLOGY

Effects of 7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide on Mitochondria from Rat Liver and Brain.—Bernard Kadenbach and Prof. Walter Lührs 174

A Non-diuretic Benzothiadiazine with Anti-hypertensive Properties.—Dr. Alan A. Rubin, Franklin E. Roth and Martin M. Winbury 176

RADIOBIOLOGY

Effect of γ-Radiation on *Escherichia coli* grown in Deuterium Oxide Medium.—Ernest Pollard . . 177

Specificity of Plant Feeding in Mosquitoes as determined by Radioactive Phosphorus.—Dr. A. A. Abdel-Malek and Dr. W. F. Baldwin 178

Effects of Irradiation on the Incorporation of Amino-Acid into Normal and Regenerating Rat Liver.—Dr. Nirmal K. Sarkar, Dr. Anima Devi and Dr. Louis H. Hempelmann 179

HÆMATOLOGY

Role of Hageman Factor in the Activation of Vasodilator Activity in Human Plasma.—Dr. M. E. Webster and O. D. Ratnoff 180

Presence of Two Iron-Transport Proteins in Serum.—Dr. M. Faber and R. Jordal 181

Genetical Evidence for Synthesis of Transferrin in the Fœtus.—Dr. A. R. Rausen, Dr. P. S. Gerald and Dr. L. K. Diamond 182

IMMUNOLOGY

Rapid Preparation of Fluorescent Antibodies using Gel-Filtration.—Johan Killander, Jan Pontén and Lennart Rodén 182

BIOLOGY

Fluctuations in Hormone Activity in Brachyura Larvæ.—John D. Costlow, jun. 183

Axenic Cultivation of an Enchytraeid Annelid.—Dr. Ellsworth C. Dougherty and Ejorn Solberg . . 184

Life-cycle of an Acanthocephalan Parasite of the Eider Duck.—Dr. C. Rayski and Miss E. A. Garden 185

ENTOMOLOGY

Intracellular *Rickettsia*-like Micro-organisms in Certain Ticks.—M. A. Roshdy 186

Biological Species confused under the Name *Culicoides austeni* (Carter, Ingram and McFie).—D. H. Murphy 186

Sorghum Midge, *Contarinia sorghicola* (Coq.), in West Africa.—K. M. Harris 187

Effect of Age and Pregnancy on the Tolerance of Tsetse Flies to Insecticides.—G. F. Burnett . . . 188

Increase in Distribution of *Deraeocoris olivaceus*.—Dennis Leston and G. E. Woodroffe . . . 188

MICROBIOLOGY

New Method of preparing *Corynebacterium pyogenes* Toxoid with High Anti-hæmolysin Titre in Rabbits.—Valerie Levy 189

Antibacterial Synthetic Polypeptide Derivatives.—Dr. K. Kovács, Dr. A. Kótai, I. Szabó and R. Mecseki 190

Nature of Colicine Receptors.—Dr. Anna Mayr-Harting 191

MISCELLANEOUS

A Simple Flattener for holding Filter Papers on Planchettes for Radioactive Counting.—Dr. Edward A. Smuckler and Prof. Earl P. Benditt . . . 191

Chemico-Linguistics: Computer Translation of Chemical Nomenclature.—E. Garfield 192

FERTILE HENS' EGGS

Large or small numbers always available, in non-returnable boxes if preferred

PART INCUBATED EGGS
WITH LIVE EMBRYOS
CAN BE SUPPLIED TO ORDER

Day-Old Chicks, Pullets, Cockerels and Hens

N. W. WITHERS
Appleby Farm
EGERTON • ASHFORD • KENT
TELEPHONE: PLUCKLEY 247

GRIFFIN
Established in 1820

THE ADVANCED THEORY OF STATISTICS

M. G. Kendall, M.A., Sc.D., Pres.R.S.S.
Alan Stuart, B.Sc.(Econ.)

VOL. I. *DISTRIBUTION THEORY*

Although a good deal of the material of the previous edition of this volume has survived, substantial additions were effected in the preparation of the present form, owing to the advances in theory.
433 pages (10"×7"). Over 200 exercises. 84s. net

VOL. II. *INFERENCE AND RELATIONSHIP*

This volume has been almost entirely re-written and rearranged, at the same time greatly enlarged. The authors' standpoint is objective and only in discussing the vexed question of the difficult approach to inference do they descend into the arena of controversy.
676 pages (10"×7"). Over 400 exercises. 132s. net

VOL. III. *PLANNING AND ANALYSIS AND TIME-SERIES*

This volume will be published in 1964.

Send for 16-page list of books and monographs on Statistics

DRURY LANE
LONDON

Resistance Wires

TOPHET 'A' — 80/20 Nickel-Chrome — gives best results in all high temperature applications.

TOPHET 'C' — Nickel-Chrome-Iron — lower priced but excellent for use at slightly lower temperatures.

CUPRON — Copper-Nickel—low temperature coefficient alloy for use at low temperatures.

BALCO — High and uniform temperature coefficient alloy.

EVANOHM — The best available material for super precision resistors. Highest specific resistance and lowest temperature coefficient.

* *For complete data on all our resistance alloys and stainless steel wire, write for a copy of our Resistance Handbook.*

GILBY-BRUNTON LTD
Head Office & Works:
SEAMILL, MUSSELBURGH, SCOTLAND
Telephone: Musselburgh 2369
London Office: 47 WHITEHALL, S.W.1
Telephone: WHitehall 6058

PRECISION BS INSTRUMENTS

Two New Refractometers

Two specialised refractometers have been built and are now offered as standard instruments in our wide range.

R.32 Employing a vee cell similar to that offered as an accessory to the Pulfrich refractometer. Intended for use with liquid samples, it has a range from 1.30 to 2.30 with an average accuracy of .0001 using conversion tables.
The principle employed is the measurement of the deviation of a collimated beam of monochromatic light through the liquid sample constrained into a prism of known angle.

R.320 For the measurement of index of a transparent liquid sample at any wavelength between 2,300 and 6,000 Å. Can be direct reading to .001 and by estimation to .0002, over the range 1.30–1.70 Photo-electric detection is employed and the instrument is open to development for recording should it be required.

Bellingham & Stanley Ltd.
DEPT. N, 71 HORNSEY RISE, LONDON, N.19
PHONE: ARCHWAY 2270
Sole Distributors in U.K. for Schott Monochromatic Interference Filters

THE AMATEUR IN SCIENCE

In an address on August 31 to Section L (Education) at the British Association meeting at Norwich, in which he surveyed some current programmes of research in education, Dr. W. D. Wall, director of the National Foundation for Educational Research in England and Wales, commented that he was himself an amateur in research ; and he raised the question of the place of the amateur in research. It is true that, in the physical sciences, the high cost of equipment has in itself largely eliminated the amateur and even the individual, but the position is perhaps too lightly accepted without due thought for the consequences. It could be argued that the creative years of an individual scientist, in which his most important contributions are made, are those early years in which he is emerging from amateur or tyro to true professional status. Again, it could be suggested that part of the value of mobility between one technology or one particular subdivision of a scientific discipline and another lies in the stimulus which a fresh outlook can supply.

To some extent this can be found in the research teams comprising scientists and technologists trained in different disciplines which are now commonly built up for the attack on problems which involve contributions from different disciplines; but it might be worth while to re-examine, under modern conditions, the contribution which the amateur could make, and the relations between amateur and specialist to-day, as well as the factors that most conduce to co-operation. There could scarcely be a more serious reproach to the system of education in Britain than the admission of a leading educationist that the first task of institutions of further education is to awake the desire to learn, and in his presidential address to Section J (Psychology) Prof. George Seth remarked that intellectual eagerness is by no means the most obvious characteristic of the university entrant of the mid-century. Prof. Seth was concerned particularly with the attitude of the student to knowledge, to learning and scholarship, and the disinterested search for truth. If, as he pointed out, a student lacks concern for these matters, even the able student will be unfitted for the university environment, and it is also true that he may be handicapped in his subsequent professional as well as in his university career.

It was therefore fitting that two of the sectional presidential addresses at Norwich should be concerned with aspects of amateurism or intellectual curiosity. Prof. Seth, who thinks that the universities may already be getting as many of the intellectual *élite* as they could expect, was much more concerned with the quality of teaching, and particularly the consequences if many of the brightest youngsters are taught largely by those who are intellectually less able than themselves. Apart from this, he suspects that some of the teaching techniques in British universities have been ill-designed to encourage or to stimulate creative originality in their students. He puts this fairly to the universities as in part their problem, though they may reasonably look to the schools for help. What is looked for is imaginative freedom rather than conformity or preoccupation with satisfying the examiners, and Prof. Seth rightly points out that such a quality can only flower in an atmosphere of freedom.

Clearly there is here a line of research that should commend itself to the universities and to their psychology departments, as well as to institutions concerned with educational research; and the problem is urgent for if, in the immediate future, we must look to the schools to stimulate this quality, the universities should be striving to acquire teachers of sufficient number and quality to meet the need in all departments. It is to the teacher that Prof. Seth looks primarily, and he maintains that an active scepticism of traditional educational instruments, institutions and philosophy holds enormous possibilities for good. A community that lives complacently on the inheritance of accumulated experience that we call tradition is already dead, however rich and splendid its past may have been; but Prof. Seth also reminds us that, without that past, and without a clear and just appreciation of its meaning for us, our future advance will be feeble, lacking in impetus, uncertain and wavering in direction.

There in some degree is expressed the case for co-operation between amateur and professional, and it might be held that the Earl of Cranbrook's presidential address to Section X on September 1 on "Science, Politics and Social Service" expounds that theme. Insisting on the importance of the possession of a curious and inquiring mind, which is regarded as a main difference between man and other animals, Lord Cranbrook notes the bearing of intellectual curiosity and the scientific outlook on the advances in the seventeenth and eighteenth centuries. He comments on the range of interest which marked the early Fellows of the Royal Society, and shows how out of such amateurism in the true sense arose the scientific societies of the eighteenth and nineteenth centuries. As a result, the gradual advance towards universal compulsory education, which was fortunately faster than the gradual extension of the franchise, was accompanied by the growth of societies which encouraged men actively to do things for themselves as well as being educated passively by the educational methods of the day.

Lord Cranbrook emphasized the majestic diversity of the thoughts and achievements of the great scientists of the sixteenth and seventeenth centuries, and

pointed out that the ordinary educated man of those days could still make his contribution to science well on into the nineteenth century, and he does not accept as inevitable any divorce between science and the humanities. He makes no ill-founded claims for political wisdom or judgment for the scientists, even for biologists or economists. Scientists are just as ready as other men to fit themselves into the Procrustean bed of their own ideology and to study the humanities with all the blind prejudices of the most obscurantist politician. The connexion between scientific and political thought to which he referred could only exist in the earlier stages of man's development, and the last great advance in scientific thought, which grew out of the Renaissance, built up a scientific discipline inapplicable to political or sociological problems. In Lord Cranbrook's view, the scientist's training and method of approaching his problem no more fits him to be the arbiter of political thought than does that of the priest, the actor, musician or author.

While Lord Cranbrook thus does not suggest that the scientist alone can put things right in the world, he certainly gives no support to the idea that the politician should know little or nothing of science or take pride in being, as Mr. Harold Macmillan claimed at the Royal Society Tercentenary banquet, the only amateur remaining. In the world of to-day, men and women in all spheres should understand what is happening and why, and the growth of leisure should facilitate this. Moreover, a well-balanced man or woman should be able to understand and enjoy, at least in part, both arts and sciences; very appropriately, since he was addressing the Assembly of Corresponding Societies, Lord Cranbrook pointed to opportunities which these societies even to-day could use to foster such understanding. The development of communal life is indeed a step to the development of political life and understanding.

Nor is this confined to the village or rural communities of Britain or Europe. Perhaps the most important passage of Lord Cranbrook's address is that in which he discusses the bearing of our experience on what is happening in the under-developed countries. The major scientific societies of those countries are unlikely to pass through the stage of consisting mainly of amateurs, as did the Royal Society and other learned societies in Europe in the seventeenth and eighteenth centuries. They will consist mainly of professional people, and the developing schools and universities of these countries may have to concentrate on producing professional men and technicians. In the immediate future medical men, veterinary surgeons, engineers, agricultural advisers and teachers are the primary need rather than research workers; but even so, it will be important that the general body of the population should understand something of the meaning and purpose of science.

Since these professional scientists are likely to be preoccupied in professional practice or the training of technologists and technicians, Lord Cranbrook suggested that it will be the more important that a body of amateurs should be built up to study the natural history, archæology and local history of these countries. He visualized a series of Associations for the Advancement of Science, from which these peoples can learn something of the pleasures as well as of the meaning and purpose of science, and he sees a very clear parallel between technical development and knowledge of natural history in Britain 150 years ago and in the less highly developed countries to-day. It was as we passed from being a mainly agricultural to a mainly industrial community that men of all classes were busily engaged on finding, describing and cataloguing the living things around them.

Lord Cranbrook thus looks to something of a renaissance of the local scientific society both in Britain and in the lesser developed countries, and in this he has the support of Sir Cyril Hinshelwood, who, in a notable address last autumn to the Manchester Literary and Philosophical Society, referred to the part which such societies could play in promoting understanding between the arts and scientists. Moreover, Lord Cranbrook recognizes that something can be done to help under-developed countries on the social side also through the technical assistance which is now being increasingly given. He did not refer specifically to either the British or the United States schemes for voluntary service by young people overseas; but, of the young people from Britain who are going to the under-developed countries in increasing numbers on medium-term engagements as technicians or teachers and for short periods to assist generally in social work, he emphasizes that it is important that they should all realize how Britain developed and how our experience might be useful to others.

Perhaps the Voluntary Service Overseas scheme may have particular potentialities in this connexion; but for any effective assistance to be given in this way by those who proceed overseas under that scheme or any scheme of technical assistance, there is one essential condition. Something more than knowledge is required—an inquiring mind and the eagerness to learn which mark the amateur and characterize the true professional are needed. Unless both are nurtured and encouraged by schools and universities alike, it is idle to expect that Britain will maintain the excellence which has characterized her scientific work and much of her technology in the past; and still less that she will make the contribution to the leadership of the emergent nations in welfare or in scientific and technical development which her experience and her tradition so richly qualify her to render. These two addresses at Norwich have pointed to opportunities and they have issued a challenge which should be accepted not only by the schools and the universities but also by many of the professional institutions, learned societies and the local societies in which professional and amateur have often come together and co-operated on terms of mutual respect and understanding. Moreover, the same challenge was issued, but in another context, quite as clearly by the Master of Sidney Sussex College, Cambridge, Dr. David Thomson, in speaking of what the universities expect from the schools. Thus he referred to the

subtle and imperceptible threshold between pupil and student, and the absence of evidence that everyone can reach or cross this threshold; and he urged that it is the supreme duty of a sixth-form teacher to lead his charges to this threshold and encourage them to venture beyond, independent of teacher or text-book. A similar task, different only in level, confronts every teacher in further education. Britain's economic and industrial future, no less than the extent to which she can rise to her opportunities of leadership, alike depend largely on the effectiveness and vision with which it is discharged.

MATHEMATICAL TABLES

Table of Values of the Function

$$w(z) = e^{-z^2}\left(1 + \frac{2i}{\sqrt{\pi}}\int_0^z e^{t^2}\,dt\right)$$

for Complex Argument

By V. N. Faddeyeva and N. M. Terent'ev. Edited by V. A. Fok. Translated from the Russian by D. G. Fry. (Mathematical Tables Series, Vol. 11.) Pp. v+280. (London and New York: Pergamon Press, 1961.) 100*s*. net.

Tables of Airy Functions and Special Confluent Hypergeometric Functions for Asymptotic Solutions of Differential Equations of the Second Order

By A. D. Smirnov. Translated from the Russian by D. G. Fry. (Mathematical Tables Series, Vol. 7.) Pp. vii+260. (London and New York: Pergamon Press, 1960.) 100*s*. net.

Bessel Functions

Part 3: Zeros and Associated Values. Edited by F. W. J. Olver. Prepared under the direction of the Bessel Functions Panel of the Mathematical Tables Committee. (Royal Society Mathematical Tables, Vol. 7.) Pp. lx+79. (Cambridge: At the University Press, 1960.) 50*s*. net.

Tables of the Riemann Zeta Function

By C. B. Haselgrove, in collaboration with J. C. P. Miller. (Royal Society Mathematical Tables, Vol. 6.) Pp. xxii+80. (Cambridge: At the University Press, 1960. Published for the Royal Society.) 50*s*. net.

Representations of Primes by Quadratic Forms

Prepared by Hansraj Gupta, M. S. Cheema, A. Mehta and O. P. Gupta. Edited by J. C. P. Miller. (Royal Society Mathematical Tables, Vol. 5.) Pp. xxiv+125. (Cambridge: At the University Press, 1960.) 45*s*. net.

ANY suggestion that the advent of electronic computers may lead to the cessation of further publication of mathematical tables is refuted by this collection of new tables. The collection is particularly interesting, since the first two are of Russian origin, and allow some sort of comparison with British tables.

The first volume deals with a function $w(z)$, which at first sight would appear to have little practical value. But if we write $z = x + iy$, then both $w(x)$ and $w(iy)$ are simply related to integrals of $\exp \pm t^2$, and when $x = y$, $w(z)$ is connected with integrals of the Bessel function $J_{\pm\frac{1}{4}}(t)$. The function itself may be expressed in the form:

$$w(z) = \frac{i}{\pi}\int_{\infty}^{\infty} \frac{\exp -t^2\,dt}{z-t}$$

It occurs in scattering theory, in the propagation of electromagnetic waves, and in the breadth of spectral lines. These tables give $w(x + iy)$ for $0 \leqslant x,y \leqslant 5$, to 6 figures, with differences, and at a tabular interval of 0·1. The tables themselves are clear to read, but it is a pity that not more information is available about their checking, and the elimination of errors. The publishers apologize for the 'messy' character of the introductory pages. The apology is very appropriate.

The second volume, by Smirnov, deals with Airy functions. These functions satisfy differential equations of the form $y''(x) + x^a y(x) = 0$. They appear frequently in all sorts of numerical methods, and their tabulation in detail is very desirable. This collection gives $y(x,a)$ and certain derivatives and differences, for $|x| \leqslant 6$ at intervals of 0·01, and for 16 rational values of a between $-\frac{3}{2}$ and 2. Lagrangian interpolation is possible to within 10^{-3} on average. The tables are beautifully printed, and are presumably copied photographically from the Russian original. The preliminary explanatory material is less attractive, and the volume is rendered less satisfying than it could be by the inclusion of some so-called "confluent hypergeometric functions", which are quite distinct from the usual function $F(a,b,x)$ of that name.

The remaining three volumes are all part of the Royal Society series of mathematical tables. Here we meet an element of distinction, previously missing. All three introductory sections are alive, well informed and interestingly written. In particular, the volume on Bessel functions—which is No. 3 in the series, and follows earlier volumes in 1937 and 1952—reads like a detective story. It is an intriguing sign of the influence of electronic computers that in the end, as part of the checking process, 18 years of work by desk machines was repeated in 12½ hours on the computer. But zeros of transcendental functions are notoriously difficult to evaluate. Here again are given the first fifty zeros of J_n, Y_n, J_n' and Y_n', together with those for the gradient of the spherical Bessel functions, for $n = 0(\frac{1}{2})20\frac{1}{2}$. The layout is good, and the methods of testing and checking are clearly stated. This is a first-rate volume.

With the Riemann zeta function we enter the realm of pure mathematics. There is still no proof that all the non-trivial zeros of $\zeta(s)$ in the critical strip for which the real part of s lies between 0 and 1, are on the critical line bisecting this strip. But this seems to be the case so far as calculations have been carried. This volume gives the real and imaginary parts of $\zeta(\frac{1}{2} + it)$ and $\zeta(1 + it)$ for $t = 0$ (0·1) 1000 to 6 decimals. Immense tabulation on a computer was needed. But the results are worth it.

Finally, we have Gupta's tables giving various representations of prime numbers p not exceeding 100,000. If $p = a^2 + Db^2$, where $D = 5$, 6, 10 or 13, the values of a and b are given (if they exist) for all p. So also is the least value of k and corresponding n, where, for the same p and D, $kp = n^2 + D$. These tables are easy to read, and should give much pleasure to those mathematicians interested in the theory of quadratic fields. The tests used by the authors are impressive. The typing of the tables in a form suitable for direct photo-copying was admirably done on a card-controlled typewriter. C. A. COULSON

XUM

ORIGIN OF THE NORTHERN RHODESIAN COPPER DEPOSITS

The Geology of the Northern Rhodesian Copperbelt

Edited by Dr. F. Mendelsohn. Pp. xvi + 523. (London : Macdonald and Co. (Publishers), Ltd., 1961.) 84*s*. net.

IN November 1902 W.C. Collier shot a roan antelope in the bush while prospecting near the Luanshya River : close to the body of the buck he found outcrops carrying copper and pegged a claim. Twenty-nine years later Roan Antelope became the first of the great Northern Rhodesian copper mines to come into production. To-day the Copperbelt supports a community of more than a quarter of a million and produces nearly one-fifth of the free world's copper.

Something of the scientific effort behind this great expansion is revealed by the twenty-three authors of the monograph under review. The majority are members of the geological staffs of the Anglo-American Corporation and Rhodesian Selection Trust, but there are also contributors from the British South Africa Company and Union Minière du Haut-Katanga. Since the ore deposits on the Copperbelt are almost wholly confined to a series of sediments of presumed Pre-Cambrian age, the Lower Roan Group of the Katanga System, the first necessity for exploration was a geological map showing the extent of these rocks. Its production was aided by aerial photography, and the recognition of individual deposits has been facilitated by a variety of geophysical and geochemical prospecting techniques.

Economic mineralization on the Copperbelt is concentrated in a zone about 100 miles long and up to 40 miles across. This is, however, but a fraction of the 500-mile arc of folded Katanga beds which stretches through Northern Rhodesia and the Congo into Angola, covering more than 80,000 square miles and forming one of the world's greatest base-metal provinces. Structurally, the Copperbelt is dominated by the north-west trending Kafue anticline, which brings up a central belt of pre-Katanga rocks and is flanked to the north-east by the Mufulira syncline and to the south-west by a series of *en echelon* downfolds on the flanks of which Bancroft, Nchanga, Chibuluma, Nkana and Roan Antelope Mines are situated. The ore-bodies occur in rock of varying lithology: in the Mufulira basin they are predominantly arenaceous, to the south-west more commonly argillaceous.

Lively controversy has surrounded the origin of the ores. On one hand their great lateral extent, their persistence at certain stratigraphical horizons, their intimate connexion with sedimentary features of the host rocks, the inferred relation of sulphide zoning to facies, the evidence that much of the mineralization is pre-folding, the absence of any obvious deep-seated source for the mineralizing solutions and the difficulty of envisaging movement of large volumes of such solutions for great distances through sediments of low permeability have suggested a sedimentary syngenetic origin. Lean copper deposits known to occur in the Pre-Katanga rocks have, it is suggested, been eroded, transported in solution to the basin of deposition and precipitated during the sedimentation of the Lower Roan Group. Subsequent redistribution has taken place during the folding and metamorphism of the sediments. On the other hand, there are many features more readily explained in terms of epigenetic hypogene mineralization. These include the relation of the deposits to the central region of the Katanga geosyncline and to fold axes, the presence of local mineralization in Pre-Katanga rocks immediately beneath ore-bodies in the Lower Roan Group, the geochemistry of the sediments, suggesting that they have undergone widespread metasomatism, and the inadequacy of the metamorphic temperatures capable of producing rocks of the greenschist facies to account for certain observed mineralogical features.

In many cases the evidence recorded from different mines is conflicting. At Roan Antelope ore has been proved over a strike length of about 7 miles and to a depth of nearly 4,000 ft. in a stratigraphically controlled ore-body with an average thickness of 25 ft. : at Nchanga it transgresses stratigraphical boundaries through more than 300 ft. of varied sediments. At Chibuluma and Roan Antelope the mineralization is unrelated to the folding : at Nkana South the ore-body is confined to the highly contorted and fissile portions of carbonaceous shales. At Mufulira mineralization is regarded as pre-dating slumping : at Mindola it appears to post-date faulting. It is scarcely to be wondered at that the various contributors fail to reach agreement as to the genesis of the ores, though more space is devoted to the sedimentary syngenetic theory than to any other. It is perhaps more surprising that no attempt is made to reconcile the puzzling association of criteria suggesting both syngenesis and epigenesis in some mechanism such as long-continued infiltration of ascending solutions into the basin of deposition as well as into the growing pile of sediments.

Supergene alteration persists to great depths on the Copperbelt, and some of the conflicting evidence may be a consequence of failure to recognize the full extent of secondary sulphide enrichment. More detailed investigation of this problem is evidently needed. Equally essential is the study of the relation of mineralization to both sedimentary and tectonic features in the base metal province of the Katanga geosyncline as a whole rather than in the small portion of it which forms the Copperbelt. Political factors, unfortunately, make this a more difficult task to-day than at any time in the past fifty years. When it can be undertaken it should prove both scientifically rewarding and commercially profitable.

J. H. Taylor

SCIENCE IN POETRY AND VERSE

A Book of Science Verse

The Poetic Relations of Science and Technology. Selected by W. Eastwood. Pp. xvi + 279. (London: Macmillan and Co., Ltd., 1961.) 21*s*. net.

THIS is a pleasant and successful anthology of the treatment of 'science'—the term is given a wide construction—in poetry and in verse. Mr. Eastwood, who is vice-principal of Reading Technical College, starts on an optimistic note. He points out in his introduction that "the separation of science and poetry does not go back much further than the late eighteenth century", that "poet and scientist are alike concerned with the ordering of experience, and to both the imagination is important" . . . "a poetry which ignores science and its applications is, in the modern world, divorced from life" . . . "There is no future for a culture which cannot reconcile the poetic

and scientific impulses, for both are necessary to the human spirit". These statements, apparently so simple, do in fact raise issues of vast importance; an appendix of selected pronouncements by Wordsworth, Peacock, Herbert Spencer, A. N. Whitehead, I. A. Richards and C. Day Lewis provides material for further argument. But the greatest difficulty of all goes unsolved: the basic and ever-increasing difference in the vocabularies of scientists and poets. John Donne could use astronomy, alchemy, metallurgy, optics and a little geometry, because knowledge had not yet given them a specialized diction. A glance at technical glossaries—say, of computers or guided missiles—offers little hope for their use in metrical structures.

Among the poems, or parts of them, that we should have expected are those from Lucretius, Chaucer, Shakespeare, Donne, Milton, Butler of *Hudibras*, Akenside, Erasmus Darwin, Wordsworth, Shelley, Tennyson, Kipling; but Mr. Eastwood has cast his net far more widely, and with effect. It is improbable, for example, that the common reader will know Blackmoor's *Anatomy and Revelation*, Young (dullest of poets) on *Scientific Theology and Religious Naturalism*, Hood's *Craniology*. He will find an extract from Blake's *Vala* and perhaps regret that he does not find more passages from, let us say, *Jerusalem*.

But it is in the poetry of our own century, and in particular of the past twenty years, that Mr. Eastwood's harvest is richest; Hart Crane's admirable *The Bridge*; Kathleen Raine's *The Human Form Divine* and *Rock*; Patrick Dickinson's *Jodrell Bank*; Robert Conquest's *Guided Missile Experimental Range*; John Wain's *Poem Feign'd to have been by an Electronic Brain*; and much else. A reviewer is prompted, inevitably, to speculate on what he himself might have added or omitted. 'Q's parody of Sir Patrick Spens is good only in parts. Surely there was a case for including W. J. Turner's *Geological*, perhaps his *Formal Lyric*; Wilfred Ashley's *Goods Train at Night*, possibly the magnificent epitaph in terms of the railway that rests in the south transept of Ely Cathedral. And while we have a poem on a tractor—there must be many written in the Soviet Union, too—why have we no poem on motor-cars?

T. R. Henn

A NEW LOOK IN PHYSICS TEXT-BOOKS

Physics

(Physical Science Study Committee.) Pp. viii + 656. 40*s*. Laboratory Guide for Physics. Pp. vii + 87. 10*s*. (Boston, Mass.: D. C. Heath and Company; London: George G. Harrap and Co., Ltd., 1960.)

THIS book represents a new kind of physics course. It is a year's work intended for American high-school pupils aged about 16 years, who may have done no physics previously and who need not necessarily be doing mathematics concurrently. It is excellent by any standards; authoritative, well planned, clearly written, and in this final (or more probably, current) edition, which is the outcome of drafts already tested in schools, well produced. It is unlikely, at least in the near future, that many British teachers will find themselves using such a course; but this is a book which every physics teacher ought to read. He will find it stimulating and instructive; it will also possibly provoke some disagreement —and it is about time that teachers in Britain had something as provocative as this to shake up their ideas.

The Physical Science Study Committee in the United States has organized this course down to the finest detail, covering text-book, laboratory guide, apparatus, films, tests, and background reading, and also the teacher who is, as Prof. Zacharias has said, only one of the range of teaching aids. Indeed, experienced teachers are being re-trained on full-time courses lasting about a term to prepare themselves to work this syllabus. This almost savours of 'The Method'; but both British and American physicists who have been to these courses assure me that after a short initial phase of scepticism they have been imbued with the ideas of the Physical Science Study Committee. It should also allay the misgivings of those who feel that the concepts of modern physics can only be presented to schoolboys in a dogmatic manner, which is the chief basis for any disagreement with the Committee's course; for it is intended to be taught by people who have been converted to the belief that it can be done soundly, scientifically, and as undogmatically as anything can be done at school—and who understand how.

The text-book is in four parts, each the work of a group composed of university physicists and school-teachers. The first part deals with the measurement of time and distance, motion, vectors, the concept of mass, elements, atoms and molecules. Part 2, on optics and waves, explains wave propagation very fully, and gives a detailed treatment of interference and diffraction; it also discusses the particle behaviour of light. Part 3, on Newtonian mechanics and gravitation, deserves a very careful study by those who are suspicious of dogma. Newton's third law (which, incidentally, is not called that) is obtained inductively (as Newton found it) from experimental observations on the conservation of momentum. The really honest reader will find himself wondering whether he has not been teaching dogmatically for years. Part 4, on electricity and atomic structure, is written in rationalized M.K.S. units; sooner or later science teachers in Britain will have to be as adaptable as teachers in other countries who must have undergone the discipline of mastering them on the job recently. But the work on electromagnetism is made crisper than usual by the elimination of the vector H. It is possible that the treatment may be revised in places. Great emphasis is laid, for example, on Millikan's experiment for e, performed with small plastic spheres. This has been criticized as taking a lot for granted, and I believe the Committee was rather worried about its soundness; but the latest report on it was reassuring. Atomic structure is dealt with entirely in terms of energy-levels, the existence of stationary states being justified by an appeal to elementary wave-mechanics. The Bohr atom itself, which looks as if it may at last be finding its way into British school syllabuses, has been dispensed with in this book.

Many of the experiments in the laboratory manual are to be commended as they show what can be done with quite simple apparatus. These include: the determination of molecular size; the examination of spectra and the determination of wave-length; the relation between inertia-mass, gravitational-mass and weight; the verification of Kepler's law of equal areas; and the determination of the mass of the electron.

XUM

Heat, as it is usually taught, does not come into the course at all; and, in what is intended for a one-year course, the compilers have had to be rigorously selective in other directions as well. They have concentrated on what they believe to be fundamental and what can be well taught. They report that the course has proved itself in practice, and the tests given to the pupils are certainly exacting. I was able to obtain a specimen set, and I can only say that I should not care to tackle them after a hard day spent determining coefficients of expansion.

Prof. Francis L. Friedman, of the Massachusetts Institute of Technology, describes the development of the text-book in an appendix, and lists the 200 or so contributors to the programme. They must be proud of their achievement. G. R. Noakes

MATERIALS FOR REACTORS

Reactor Handbook, Vol. I

Materials. Edited by C. R. Tipton, Jr. Second edition, revised and enlarged. Pp. xv+1207. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1960.) 260*s*.

THE first edition of this handbook was published by the United States Atomic Energy Commission before the 1958 Geneva Conference, at a time when little atomic energy information had been released. It is not surprising, therefore, that considerable expansion and revision of the data have been necessary in such a rapidly expanding field and with the declassification of much of the earlier material. In particular, both liquid and gaseous reactor materials have now been included and the number of authors contributing extended to some 130 reactor specialists. The majority of the data has been drawn from U.S. Atomic Energy Commission reports, many of which are still classified, with generally only brief mention of British and other European work. It is claimed that the subject-matter is current to about the spring of 1958, which would suggest that little of the information released at the 1958 Geneva Conference is included.

It is only possible to indicate briefly the scope of a volume of 1,200 pages which covers a wide range of materials. In a general introductory section, brief surveys are made of radiation damage and of the health and safety aspects involved in handling the special materials in the nuclear energy industry. About a third of the book is devoted to the production and properties of fuels, which include those based on uranium, plutonium and thorium metals both in solid and liquid form and as ceramics, aqueous solutions and molten salts. A most comprehensive list of canning and control materials is considered and the moderators reviewed are hydrogen-containing compounds, graphite and beryllium-base materials of interest. The properties of both gaseous and liquid metal coolants are detailed together with compatibility data relevant to reactor operation. A section on shielding materials covers their physical and mechanical properties and behaviour under irradiation. The volume concludes with a selected list of references dealing with the constitution of various binary alloys, which is in addition to a large number of equilibrium diagrams of technological interest distributed throughout the text.

Certain aspects of reactor technology of somewhat limited interest are described in considerable detail, whereas other problems, the elucidation of which has occupied a great deal of effort, are not dealt with adequately. For example, swelling of uranium metal fuels which can severely restrict their life under certain operating conditions, is barely mentioned, and the section on radiation damage in beryllium could justifiably be expanded. This also applies to the problems associated with pressure vessel steels, which could be considered in much greater detail. The section on magnesium alloys only briefly mentions the magnox series which were developed by British workers and adopted as the canning material for the first seven Central Electricity Generating Board nuclear power stations and ignores the zirconium-bearing *ZA* alloy which the French have developed successfully for their reactors. One is left with the impression that the handbook was prepared to satisfy the interests of United States reactor technologists up to 1958 rather than to present a more comprehensive and general survey of reactor materials. Nevertheless, it is a valuable reference book and contains a wealth of useful information.

B. W. Mott

ENZYMES AND CANCER

Chemistry of Enzymes in Cancer

By Prof. Franz Bergel. (A Monograph in American Lectures in Living Chemistry.) Pp. xi+122. (Springfield, Ill.: Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1961.) 44*s*.

THIRTY years ago biochemists were hopefully seeking for qualitative differences in metabolism between normal and tumour cells which might explain their radically different biological behaviour. That search is presumably over, and we are left with only a few quantitative differences, most of them of dubious relevance to the problem of the causation of cancer.

Prof. Bergel starts from the working hypothesis that these differences, which are the outward signs of changes in the deoxyribonucleic acid constitution of the cells, are related to the abnormal properties of cancer cells, and that if the metabolic differences could, by any therapeutic means, be removed, then the cells would revert to normal behaviour.

In Part 1 he outlines the three major kinds of hypothesis which attempt to relate enzymes to cancer: (1) the 'faulty' respiration theory of Warburg; (2) the generalization of Greenberg that all tumours possess a similar enzymic constitution; (3) the 'deletion' theory, due chiefly to Potter, according to which the cancer cell has lost certain protein constituents, probably concerned in the synthesis of enzymes; and he criticizes each in the light of recent biochemical developments. There follows a detailed discussion of the present state of knowledge with respect to certain selected groups of enzyme systems, namely, those concerned with nucleic acid catabolism, which are deficient in tumours; those concerned with protein and amino-acid catabolism, which are deficient at least in some tumours; and the enzymes of carbohydrate breakdown, where, among many other differences, phosphorylase is deficient. All these changes are in the direction of providing an increased potential for cell division.

Part 2 deals with the possibilities of acting on tumour cells *in vivo* in such a way as to correct the faults in their metabolism and restore them to

normality. The final chapter gives several examples in detail, the emphasis being on 'restitution' therapy, in which enzymes deficient in the tumour are injected into the animal and effects observed on the rate of growth of the tumour. Cessation of growth has been observed as a result of injection of both xanthine oxidase and ribonuclease.

This is not a book for the general reader; it assumes a biochemical background, and an intimate knowledge of a vast literature. Prof. Bergel almost accepts (p. 81) that the fundamental difference between normal and cancer cells must lie in the chemistry of their deoxyribonucleic acids (DNA), and that the changes in enzyme concentration which are observed must necessarily result from this change in the DNA, through mechanisms the study of which is still in its infancy. It follows that attempts to correct enzyme imbalance by 'restitution' therapy cannot restore cancer cells to normality, though its continued application could conceivably control their harmful activities. The attempt to "repair biochemical lesions residing in the DNA" (p. 82) lies far in the future. Enzyme therapy obviously depends on the possibility of introducing macromolecules into cells. The only mechanism so far suggested for this process is pinocytosis (the engulfing of small quantities of extra-cellular fluid by cells), a phenomenon about which little is known with certainty; Prof. Bergel agrees that it is "still a subject of speculation and research" (p. 58).

The subject-matter is presented with exemplary clarity, and the book excellently produced and well indexed. It will obviously be read with the greatest interest by all those concerned in cancer research.

L. H. STICKLAND

PHYSICAL OCEANOGRAPHY

Physical Oceanography

By Prof. Albert Defant. Vol. 1 : pp. xvi+729. Vol. 2 : pp. viii+598. (London and New York : Pergamon Press, 1961.) £10 10*s*. per set of two volumes.

IN these two volumes Prof. Defant has attempted the seemingly impossible task of covering single-handed the whole field of physical oceanography. That he has succeeded with such thoroughness and in such minute detail reflects the wide range of his lifetime's work in this subject. In almost every chapter, references are found to significant original contributions by Prof. Defant himself, and the text is often illuminated with examples drawn from the results of the *Meteor* expedition of 1925–27, in which he took part.

Each volume is in two parts ; the first part of Vol. 1 deals with the properties of sea-water and related topics. Following a brief chapter on the dimensions of the oceans and the topography of the sea-floor, the next five chapters, occupying some 200 pages, deal with the physical and chemical properties of sea-water, the distribution of temperature, salinity and density in the ocean and their variation with time, and the temperature–salinity relationship and its connexion with mixing processes. These are followed by two chapters on the water budget of the Earth, and ice in the sea.

The second part of Vol. 1 is devoted to dynamical oceanography, the movement of sea-water in the form of ocean currents. After considering the forces acting on the water, and the basic hydrodynamic equations, the theory of currents in homogeneous water is developed. This is followed by a discussion of the conditions at the boundary surfaces of water masses, and of currents in a non-homogeneous ocean. Special consideration is given to currents in straits, and after a brief discussion of the effect of wind on the mass distribution and density currents, the remainder of the volume, amounting to 150 pages, is devoted to an account of the general circulation of the oceans.

In Vol. 2, Prof. Defant is concerned with periodic movements in the ocean. The first part deals with the theory of surface waves, methods for their measurement and analysis, and theories of their growth and propagation. Waves in shallow water and near coasts, long waves in canals and standing waves in enclosed basins are also considered. The second part of this volume is an account of the tides and tidal currents. The tide-generating forces, the theory of tides, and the methods of harmonic analysis of tidal observations are described first, followed by a detailed account of the tides in the Mediterranean and the shallow seas. Tides in estuaries are next dealt with; then follows an account of tides in the deep oceans, particularly in the Atlantic. There is a chapter on Earth tides and the effect of friction due to tidal currents, and the volume is concluded with a substantial chapter of 50 pages on internal waves.

The manuscript of this book was completed in 1945, but post-War difficulties prevented its publication until 1961. Several revisions had been made by then, and also the text had been translated from German to English. Inevitably there is some unevenness in the revision ; some topics have been brought up to date as far as 1957, others have been relatively neglected. The claim in the preface that "the oceanographic literature has been considered in its entirety to the end of May 1957" seems exaggerated. The first chapter on the structure of the sea-floor is out of date, and post-War revisions have left some confusion. Three different values are quoted for the maximum depth in the Pacific. Much recent work on the topography of the North Atlantic, and the geophysical structure of the Earth's crust beneath the oceans and continents could have been included. In the chapter on properties of sea-water, no mention is made of its electrical conductivity, the precise measurement of which is used extensively nowadays as a substitute for salinity determination. Post-War work in the United States on forecasting ice conditions is not referred to in the chapter "Ice in the Sea". Recent measurements of radiocarbon concentration in sea-water and its implications in estimating the time-scale of the deep circulation are not considered, and much more recent work on the generation and propagation of surface waves could have been mentioned. Besides this lack of uniformity in up-to-date revision, the text is marred by a number of misprints, especially in authors' names. But Prof. Defant disarms criticism with his quotation from Newton, that these volumes "may be read with forbearance, . . . not so much with the view to censure, as to remedy their defects" ; and indeed these errors and omissions seem minor in comparison with the vast quantity of information presented here.

Physical oceanographers may consider themselves fortunate to have so much of Prof. Defant's knowledge and experience made available to them in these volumes, even at the price of ten guineas.

J. C. SWALLOW

XUM

Priests and People

A Study in the Sociology of Religion. By Conor K. Ward. (Social Research Series.) Pp. xi+182. (Liverpool: Liverpool University Press, 1961.) 25*s*. net.

THIS book is the fruit of fifteen months field work by a team of sociologists in a Roman Catholic parish in Liverpool. It provides, therefore, a very thorough examination of the life and activities of the parish and makes available much material which can be used in assessing the effectiveness of a parish in an urban setting. This is perhaps where the chief interest of the book lies for the general reader. For more than a thousand years the territorial parish with its church and resident clergy has been the normal centre of Christian life and in the past it developed into very much more than a centre for worship only. Around the parish church was collected the educational, social and sometimes political life of the area. But with the coming of the Industrial Revolution all this was changed and the parish ceased to be the centre it had been.

The question has been raised whether the system of territorial parishes is the most effective expression of Christian community in an industrial society. Ought the Church not to be training its man-power for specialist ministries, industrial chaplaincies, etc., rather than for parochial work? Such questions are constantly being argued and strong opinions are expressed. Dr. Ward's book makes it abundantly clear that more sociological research is a first essential for finding effective answers for these and other problems. It would be dangerous to make generalizations from this study of one parish. Few parishes will be as socially monochrome as this one was. Studies of other parishes would be useful to test how far the situation here described is typical. One suspects that the social cohesion of Liverpool with its history of religious intolerance might not be so true of parishes in other parts of Britain. Yet Dr. Ward has made one thing clear. Though changes must be made to adapt the parish more effectively to its urban environment, it will not be superseded in the foreseeable future. J. Robinson

Handbuch der Astronautik, Lieferung 1–6

Herausgegeben von Prof. Karl Schütte und Hans K. Kaiser. Band 1, Heft 1: Pp. 1–32. Band 1, Heft 2: Pp. 33–64. Band 1, Heft 3: Pp. 65–96. Band 1, Heft 4: Pp. 97–128. Band 1, Heft 5: Pp. 129–160. Band 1, Heft 6: Pp. 161–192. (Konstanz: Akademische Verlagsgesellschaft Athenaion, Dr. Albert Hachfeld, 1958 und 1960.) 1.50 dollars each part.

THIS *Handbook of Astronautics* will consist of 4 volumes, each made up of about 12 sections, the individual sections being issued separately and each containing 32 pages. Of the proposed 48 sections it is the first 6 which are the subjects of this review. There is no editorial introduction to explain the aims or scope of the *Handbook*, but the intention appears to be to cover the whole subject of astronautics, from the details of rocket engines to the niceties of space law, in a series of articles of about 20 pages each, which begin from first principles and presumably in later issues will go into the subjects more fully. The issues under review contain 8¼ articles. Willy Ley writes on the history of the idea of space flight; W. Schaub on "Gravity and Its Conquest", dealing with the fundamental dynamics; J. C. Cooper on "Flight-space Law"; and A. J. Zaehringer on solid-propellant rockets. H. Stümke describes how to calculate the flight performance of unmanned rockets in considerable detail, S. J. Smith and G. P. Sutton review heat-transfer problems in liquid-propellant rocket motors, R. P. Haviland outlines the applications of satellite vehicles, and J. Eugster investigates the dangers to space travellers from cosmic rays and other radiations. Finally W. Alvermann and W. Lohse begin to discuss the theory of rocket engines, but suffer a premature cut-off in mid-sentence. These articles form sound and useful introductions to their subjects, and the *Handbook* as a whole promises to be valuable as a work of reference and for teaching purposes, though it remains to be seen whether the choice of subjects for articles will be well balanced. The book follows the unsatisfactory dual-language convention, with about half the articles in English and half in German. Since most readers are not equally fluent in both languages, the value of the book is, for them, halved. The separate sections have attractive paper covers, but are presumably intended to be bound together, since they are apt to finish in mid-sentence; so the total cost of the 1,500-page *Handbook* will be 72 dollars plus binding costs. D. G. King-Hele

A Statistical Model for Chemists

By Edward L. Bauer. Pp. x+156. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 4.75 dollars.

THE author in his preface describes this book as "a manual for the working chemist" and no more exact definition of this excellent little handbook could possibly be given. Shorn of all complicated mathematical functions and lucid in its approach, this volume supplies the chemist with typical examples taken from ordinary laboratory data, and indicates those benefits relating to economy, accuracy and interpretation which can accrue from applications of the statistical method.

The relation of the Gaussian normal distribution curve to the 'Student's *t*' concept of Gosset is clearly described, the latter function being of most general use to the chemist, who is usually concerned with the averages of small sample results.

Conventional statistical terms such as precision, accuracy, average deviation, variance, standard deviation, etc., are all concisely defined. Fixed, random and mixed models of the analysis of variance technique are illustrated by reference to three chemists carrying out certain analytical observations. Most of the tests of significance used in the book are based on the analysis of variance method, but attention is directed to the simplifications resulting from the use of range rather than variance in the calculations. Chapter 6 contains an interesting exposition of regression analysis involving correlated variables, and is illustrated by the effects of unknown 'blank' titres, etc., in quantitative analyses. Theories of sampling, control of routine analyses and factorial experimentation are all given fairly full treatment.

The volume ends with a collection of useful statistical tables, for example, for use with F and t functions, computation of confidence limits, etc.

This book should prove a most useful elementary introduction to the amateur statistician, particularly in the chemical field. D. T. Lewis

ARSENIC CONTENT OF NAPOLEON I's HAIR PROBABLY TAKEN IMMEDIATELY AFTER HIS DEATH

By Dr. STEN FORSHUFVUD, Dr. HAMILTON SMITH and Dr. ANDERS WASSÉN

Vasagatan 33, Göteborg C, Sweden, and Department of Forensic Medicine, The University, Glasgow, W.2

IT has generally been deduced from the report of the post-mortem dissection dated St. Helena, May 6, 1821, and signed by the British medical officers, Thomas Shortt, Archibald Arnott, Charles Mitchell, Francis Burton and Matthew Livingstone, that Napoleon I's death the preceding day was due to extensive cancerous lesions of the stomach.

The view that Napoleon died of 'cancer' was not accepted by Francesco Antommarchi, the man who actually performed the autopsy, who had been Napoleon's household physician for the last twenty months, and was the only physician on St. Helena with a pathologist's training. He maintained that the hepatitis the Emperor had suffered from for a long time was the cause of death. Furthermore, the Emperor's enlarged, tender liver, jaundiced complexion and yellow conjunctivæ had also been diagnosed as signs of severe hepatitis by Barry O'Meara, Napoleon's household physician during his first three years in exile, and John Stokoe, who had attended Napoleon for a week in January 1819.

Consequently, apart from Dr. Arnott, who assisted Antommarchi during the last few weeks when the morbid picture was especially baffling, those medical men who personally had attended Napoleon during his illness refused to admit that he had died from cancer.

Many medical writers with doubts about the cancer diagnosis have attempted to identify Napoleon's disease on St. Helena by analysing compilations of his signs and symptoms. This has produced surprisingly disparate results. Thus, when exiled on St. Helena, Napoleon is alleged to have had, or suffered from the sequels of, the following diseases: peptic ulcer, intestinal ulceration, various liver inflammations, undulant fever, malaria, dysentery, rheumatoid arthritis, heart failure, congenital extremely slow blood circulation, epilepsy, tuberculosis, pleurisy, severe hormonal imbalance leading to obesity and impotence (dystrophia adiposogenitalis), syphilis, gonorrhœa, intoxication from defective teeth (so-called focal infection), gout, piles, and a constitutional predisposition to severe constipation which, it is said, was fatal owing to auto-intoxication and poisoning by laxatives.

If a suitable selection is made from the variety of signs and symptoms manifested by Napoleon on St. Helena, it is a simple matter to make out a convincing case for every one of these diagnoses in turn. But if what, after all, was a fairly unchanging disease pattern on St. Helena is taken as an entity, and allowance is made for all the signs and symptoms and their interrelations, then one cannot escape the impression that all the pieces form an orderly picture of two highly characteristic syndromes, namely, the chronic and acute types of arsenic poisoning.

Napoleon's condition was rather poor during the Hundred Days. However, his health improved while he was being taken into exile on board H.M.S. *Northumberland*; and Napoleon's general health remained good during the initial period on St. Helena when, with his sole companions and collaborators, the Chamberlain Las Cases and his son, he lived in a simple cottage on the estate, 'The Briars'.

Soon after moving to 'Longwood', which became his permanent residence, Napoleon's ailments again became manifest. He turned moody, had various aches and pains, the lower legs swelled up and would not carry him, exanthemata broke out, particularly on the legs, his sleep was abnormal with either insomnia or somnolence, diarrhœa and constipation alternated, headaches occurred with increasing frequency and severity.

His indispositions at first lasted only a few days at a time, his mental powers returning undiminished in the intervals. Nevertheless, members of his staff noticed marked changes in his expression and increasing difficulties in locomotion. Napoleon's legs were swollen and often collapsed under him.

On May 1, 1816, Napoleon had an attack lasting for a whole week. Las Cases mentions that he complained of weak legs, headache, hypersensitivity to light, and felt cold and shivery; his facial expression had changed markedly, he spoke sluggishly, and was morose and taciturn. Napoleon believed himself to have gout, suggesting that his feet were swollen and painful. Though Napoleon's teeth were perfectly sound, O'Meara recorded on June 16, 1816, that his patient had toothache. Later it turned out that the toothache was due to 'scurvy', that is, stomatitis. On July 26, 1816, Napoleon experienced the first stab of that pain in the hypochondrium which was a constant source of distress through the rest of his life. After a number of brief attacks of malaise, Napoleon fell ill for six weeks in succession on October 1, 1816. A troublesome cough was now added to the list of symptoms; he had pustules on the lips as well as in the oral cavity and throat. Napoleon was tormented by an insatiable thirst and had become perceptibly hard of hearing. Lassitude alternating with restlessness was a characteristic feature. He always felt cold and liked to sit near the fireplace. He could scarcely walk, because the swollen and weak legs would not carry him. The gums swelled up and the teeth became loose. Diffuse nausea with a predisposition for spasmodic vomiting without actual regurgitation of food were also noted. Napoleon had a relapse with the same manifestations as before on December 3 and 4, 1816, and his companions now became aware of his very jaundiced complexion. On December 14, 18 and 28 there were further attacks the symptoms of which included spastic involuntary

movements and unconsciousness on several occasions. According to the head valet, Marchand, one of these bouts was combined with dysentery (that is, severe diarrhœa). For the first time in two months Napoleon went out of doors on January 26, 1817. Apart from brief indispositions in May, June and July, Napoleon's health seemed to remain comparatively good until September 25, 1817, when a period of illness set in which lasted approximately a whole year. The signs and symptoms were on the whole the same as before, but new features had been added. For example, in Dr. O'Meara's case notes for October 1817, we read: on the third a swelling was palpable on the right side near the liver, on the ninth the pains in the legs and neighbourhood of the liver had increased and pains in the shoulder supervened, on the eleventh the patient was seriously distressed by tachycardia. Afterwards severe constipation developed, the skin and conjunctivæ were yellow and the appetite was poor. The vomiting tendency was now accompanied by stomach-ache. The stomatitis was very distressing and on several occasions caused the cheeks to swell. Constant phenomena were 'febrile attacks' at nightfall and profuse sweating at daybreak. The tongue was coated, the pulse often very rapid and irregular.

Although Napoleon now had no physician in attendance—the British authorities had ordered O'Meara away from 'Longwood' on July 25, 1818—his health underwent a gradual improvement in the latter half of that year. Some symptoms nevertheless persisted for another few months, such as lancinating hypochondrial pains and painful and weak legs. Often the feet were ice cold and had to be warmed with hot towels, and the ex-Emperor frequently sat before the fire with his feet in a flannel bag.

In the final months of 1818 it seemed as though Napoleon was well on the way towards full recovery. Once more he began to take interest in his literary work and in his garden. He started taking regular exercise, a sign that his legs would support him again. In the beginning of November 1818 Bertrand, le Grand-Marechal du Palais, wrote in his diary: ". . . le malheur a passé sur son caractère, comme sur un roc, sans l'abattre et sans y laisser de traces".

But Napoleon again became seriously ill towards the end of December 1818. The manifestations included tachycardia, chills, fever, and the old trouble from the legs. In the night between January 16 and 17, 1819, his life was threatened, he lost consciousness several times, and Dr. Stokoe was summoned. (He had been asked to become the Emperor's household physician the week before.) In his bulletins, Stokoe stated that he had found the patient in a state of extreme exhaustion, his complexion was yellow and he had pains in the region of the liver and in the shoulder. There was excruciating headache, vertigo and fainting fits. Some improvement took place when the patient began to sweat profusely. Similar attacks recurred the next night and the next night but one. Stokoe issued three bulletins about Napoleon's health, and these so displeased the Governor, Sir Hudson Lowe, that Stokoe was constrained to leave St. Helena. So on January 22, 1819, Napoleon again found himself without a physician. His health nevertheless improved, and in the latter half of 1819 he was busily at work every morning from 4 a.m. to 10 a.m. supervising the extensive landscaping projects he was undertaking in his garden. He could now walk with a stick, had recovered his former good temper, and conversed pleasantly and cheerfully with his companions. Apart from a few brief indispositions, this state of good health lasted for fifteen months.

However, a new long period of uninterrupted ill-health commenced on September 18, 1820. This period was marked by at least six intercurrent attacks of violent illness with severe and acute symptoms, but during the intervals of remission Napoleon's health improved day by day even though some of the symptoms persisted. During these periods of convalescence he could be up and about and also, albeit with tottering steps, go for short strolls in the garden.

According to Dr. Antommarchi's case notes, the acute attacks were accompanied by the following symptoms: headache; dyspnœa; weak, rapid and irregular pulse; ice-cold feet and legs; gastric pains; loss of appetite; somnolence that seemed refractory to treatment; yellow conjunctivæ; yellow complexion; excessive paleness; dark rings under the eyes; coated tongue; burning thirst; tachycardia; tremor; nervous dry cough; vertigo; vomiting; sensation of heat in the viscera; pain in the legs and from the liver, sternum and shoulder regions; constipation and diarrhœa; spasmodic contractions of triceps; remarkably weak legs; restlessness; nightmares; exanthemata; loose teeth; bleeding gums; insomnia; hardness of hearing; extreme hypersensitivity to light; impaired vision; very severe general exhaustion.

On March 22, 1821, Napoleon's disease changed character, with constant severe vomiting supervening. This was doubtless because he had been prescribed and—without knowing it—taken tartar emetic. Accordingly, antimony poisoning was now an added complication. A remission lasting for two and a half weeks occurred in the beginning of April 1821, and Napoleon took this opportunity to write his last will and testament.

Late at night on April 24, 1821, there was a severe relapse with typical manifestations of acute antimony poisoning. Additional relapses occurred over the next few days. However, a moderate improvement seems to have taken place on May 3. In the afternoon of that day Napoleon unknowingly took a large dose of calomel. Thirty minutes before midnight he had copious stools with the appearance of tar. Additional evacuations of blood occurred on May 4. He died on May 5, 1821, after lying absolutely motionless for approximately 24 hr.

At the post-mortem examination the stomach was full of black blood and the gastric mucosa was very badly corroded. These findings were probably sequels of the calomel given on May 3 rather than of arsenic. On the other hand, the following signs of arsenic poisoning were encountered: enlarged and hardened liver, enlarged spleen, fluid effusions in the pleuræ, bronchial and mediastinal lymph nodes degenerated and in suppuration, loss of all body hair, large fat deposits under the skin and in the abdomen.

Napoleon was not embalmed. Yet, when his coffin was opened in 1840, those present were astonished at finding him extremely well preserved.

In the light of the foregoing, it seems that on St. Helena Napoleon suffered from chronic arsenic poisoning with intervening periods of acute arsenic poisoning. In such circumstances, we deemed it interesting to apply the activation technique in a study of Napoleon's hairs from the period on St.

Helena. Through the courtesy of M. le Commandant Henry Lachouque, the great French expert on Napoleon's life and the organizer of the permanent Napoleonic exhibitions on St. Helena and in the French Army Museum, hair taken from Emperor Napoleon I's head, probably on the day after his death, has been made available to us.

The hair sample thus obtained was sent to the Department of Forensic Medicine at the University of Glasgow, where it was examined using an activation analysis technique (Smith, 1959) as follows.

The hair sample was weighed (1·72 mgm.) and sealed in a polythene container. It and a standard arsenic solution sealed in a silica ampoule were irradiated by thermal neutrons for one day at 10^{12} neutrons/cm.^{2}/sec. in a nuclear reactor at the Atomic Energy Research Establishment, Harwell. Thereafter the sample was returned and the arsenic extracted with added carrier arsenic by a modified Gutzeit technique. The activity from the hair sample was compared with that from the standard arsenic sample and the arsenic content of the hair was calculated.

The value found for the sample of hair was 10·38 parts per million. This is high by comparison with the normal mean arsenic content of about 0·8 p.p.m. Unfortunately, it was not possible to make any distribution studies as no further hair samples were available. It is impossible to tell from the value alone whether the arsenic was evenly distributed (as expected in continuous exposure) or located in one point (as would be the case in a single large exposure).

This investigation shows the great advantage of activation analysis when only very small quantities of sample are available.

BIBLIOGRAPHY

Antommarchi, F., *Les derniers momens de Napoléon* (Paris, 1825).
Arnott, A., *An Account of the Last Illness*, etc. (London, 1822).
Beckmann, K., "Krankheiten der Leber", in *Handbuch der inneren Medizin*, **3**, 2 (1953).
Bertrand, H.-G., *Cahiers de Sainte-Hélène* (Paris, 1949, 1951, 1959).
Boutron *et al.*, *Annales d'Hyg. pub. et Méd. leg.*, **25**, 11 (1841).
Cabanès, *Au chevet de l'Empereur* (Paris, 1924).
Cabanès and Nass, *Poisons et Sortilèges* (Paris, 1903).
Douris, R., *Toxicologie moderne* (Paris, 1951).
Forshufvud, S., *Le drame de Poison à Sainte-Hélène* (Plon, Paris, 1961; in the press).
Forsyth, W., *History of the Captivity*, etc. (London, 1853).
Funck-Brentano, F., *Le drame des poisons* (Paris, 1920).
Gigon, A., "Krankheiten der Mundschleimhaut", in *Handbuch der inneren Medizin*, **3**, 1 (1953).
Gonnard, Ph., *Lettres du Comte et la Comtesse de Montholon*, 1819–1821 (Paris, 1906).
Gourgaud, G., *Journal de Sainte-Hélène* (Paris, 1947).
Gril, E., *La Marquise de Brinvilliers Empoisonneuse* (Paris, 1933).
Hansen, F., and Moller, K., *Acta pharm.*, **5**, 135 (1949).
Heffter, A., *Deutsch. med. Wschr.*, **47**, 853 (1921).
Kalima, T., *Acta chir. scand.*, **72**, 1 (1932).
Kemble, J., *Napoleon Immortal* (London, 1959).
Kratter, J., *Lehrbuch der gerichtlichen Medizin* (Stuttgart, 1912).
Las Cases, E., *Mémorial de Sainte-Hélène* (Paris, 1823).
Liebegott, G., *Deutsch. med. Wschr.*, **74**, 855 (1949).
McNally, W., *J. Amer. Chem. Assoc.*, **39**, 826 (1917).
Marchand, L., *Mémoires de Marchand*, **2** (Lachouque, Paris, 1955).
Mayers, M., *Arch. Indust. Hyg.*, **9**, 388 (1954).
Merkel, H., "Die Magenverätzungen", in *Handbuch der spez. path. Anatomie u. Histologie* (Henke and Lubarsch, Berlin, 1926).
Montholon, Ch., *Histoire de la Captivité*, etc. (Leipzig, 1846).
Munch-Pedersen, C. J., "Polyneuritis", in *Nord. läbrobok i internaed*, **5** (1958).
Nass, L., *Les empoisonnements sous Louis XIV* (Paris, 1898).
O'Meara, B., *Napoleon in Exile* (London, 1822).
Petri, E., "Path. Anatomie und Histologie der Vergiftungen", in *Handbuch der spez. path. Anat. u. Hist.* (Henke and Lubarsch, Berlin, 1930).
Saint-Denis, L., *Souvenirs du Mameluck Ali sur l'Empereur Napoleon* (Paris, 1926).
Smith, H., *Anal. Chem.*, **31**, 1361 (1959).
Wätjen, J., *Virchows Arch. path. Anat. u. Phys.*, **256**, 85 (1925).

TEACHING MACHINES

So many members were interested in the symposium on "Teaching Machines" in Section J (Psychology) at the British Association meeting at Norwich on September 4 that the 'house-full' notice had to be used. Introducing the subject, the chairman, Prof. H. Kay, pointed out the theoretical and practical implications of teaching machines. It is likely that their use in research will contribute considerably to our understanding of learning processes. Psychologists recognize that hitherto they have carried out extensive experiments on animal behaviour but have neglected the study of human learning. Here is a definite opportunity to examine in ideally controlled conditions the variables which are influencing learning. On the practical side there is not only a desperate national need for teachers, particularly in science subjects, but also an increasing problem of how to impart technological information on a world-wide scale. The urgent need is for new forms of communication; teaching machines represent one such form.

Dr. J. Annett, of the Department of Psychology, University of Sheffield, outlined the main requirements for a teaching machine. It is a device not only for imparting information but also for sensing that this information has been received and understood. It is a two-way communication channel designed at every stage to check the progress of the student and, ideally, to modify its instructions on the basis of the students' responses. This may be achieved by several methods. Perhaps the simplest is the small step or linear-type programming developed by Prof. Skinner at Harvard, where a series of statements and questions are built up and are repeatedly modified on the basis of the actual success of students in mastering them. Eventually a sequence is established so that when this programme is presented to other students nearly all the problems of subject-matter presentation have been anticipated. Such a programme is time-consuming to prepare, is lengthy in so far as there are many items with short steps between them, but is efficient and requires little use of 'gadgets' for presentation.

An alternative system is known as intrinsic programming. Here there is a main line of communication, and provided a student can respond correctly he can proceed smoothly along this route. But if he makes a mistake he is directed to a branch line, where further explanations are given before returning him to the main sequence. Dr. Annett explained that in the type of machine constructed at the Department at Sheffield this system had been achieved by a sequence of '*A*' frames and '*B*' frames. If any one of the '*A*' frames is misunderstood, a '*B*' frame is selected and the point is explained in greater detail. This type of machine is flexible enough to cope with students differing considerably in intellectual ability. A machine might operate on a

XUM

'constructed response mode' where the student first makes his own answer and compares it with the answer which is then supplied by the machine, or, on an alternative procedure, where the machine first supplies a multi-choice solution and the student has to select the correct response. The advantages of these procedures are being investigated.

Mr. Leslie Reid, lecturer in psychology in the University of Aberdeen, discussed and actually produced the simpler forms of teaching machines which he has developed and is using at present in his research work in Scottish schools. His programmes have followed Skinner's principles with an accent on eliminating frustration and difficulties. From this point of view, teaching machines preserve the teacher–pupil relationship by ensuring that pupils are consistently rewarded, and not punished, for their responses. This is achieved primarily by the ordering of items in a suitable sequence, such that the transition from ignorance to knowledge is not a bumpy and apparently unmapped journey, but a smooth and logical progression in which a pupil can be expected to achieve success. Mr. Reid explained how a pupil working through a programme achieves insight through a gradual understanding of his subject; by contrast, that insight which a pupil achieves by a sudden flash of comprehension—the so-called 'eureka' phenomenon—is mainly a product of bad teaching.

Mr. Gordon Pask, of System Research, explained the principles of an adaptive teaching machine. Unlike a programmed machine the adaptive device itself 'learns' about the student's behaviour and modifies the training routine to suit each individual throughout the learning process. In teaching a manual skill the designer has to select certain parameters. These refer both (1) to the students' behaviour, such as the response latency, and (2) to the instructor's behaviour, such as the amount of 'cue' information with each category of problem. It will be observed that the parameters in (1) define measures of the student's behaviour which an instructor might make, and those in (2) consider the ways in which the instructor might manipulate a training routine. Thus the designer is constructing a computing device which measures the performance and then by an adaptive servo-mechanism adjusts the parameters of (2) in order to increase to a maximum the performance measure. Well-designed machines adopt a competitive strategy when the student is doing well and a co-operative strategy when he is making no improvement. Mr. Pask went on to describe how adaptive teaching machines might be used for group or classroom instruction. Here students are carrying out exercises in scientific inference and act either as a problem poser or a problem solver. The student coming quickest to the right conclusion using the minimum of evidence is most successful. A miniature economy is imposed on the group, and the machine controls the group's behaviour by modifying the parameters of the game. The most useful parameters seem to be 'cost' of communication, 'cost' of evidence, 'gain' for successful solution, and a bias on the process of role assignment.

Mr. K. Austwick, of the University of Sheffield, presented "An Educational Viewpoint" on the subject. Mr. Austwick has conducted preliminary experiments at schools on the teaching of mathematics with a simple linear programmed procedure. Preliminary results are encouraging and suggest that work learned by this method is well retained over a period of time. Programming of school and university courses is now under way. If such procedures were widely adopted they could lead to modifications in school design since they do not require the formal arrangement of a classroom. The possibilities of such machines to handicapped children, isolated children, personnel in the Services, and adults keeping up to date in a subject were brought out. On the other hand, the machines could not take over the 'human' role of the teacher, though they might give the teacher more time and opportunity to show his humanity.

The discussion was brisk enough and ranged from the university teacher anxious to discover how far machines could take over the part of tutor to a self-styled 'plain local family doctor' who knew they could not. These are early days for the teaching-machine movement, but it was apparent that we have here an educational development which is already arousing considerable interest and some opposition—both happy indexes. In a remarkably short time this type of instruction has now used 'hardware' ranging from simple pencil and paper booklets to electronic gadgets controlled by a full-scale computor. Systems are already being produced for public sale by both the publisher and the electronics industry. It is clear that the results of research are needed to give some guidance to this diversity, but research efforts in Britain are, as usual, on a small scale. To the academic psychologist this subject represents a further development in the field of communication systems, and it now offers another opportunity for studying how man can most efficiently receive and store information. But the pace and detachment of academic research are confronted by the urgency of the demand for instruction. The need in Britain for scientific and technical teaching is obvious enough, but is trivial in comparison with other areas of the world. Part of the impetus behind automated teaching is that universally, in so many subjects, there is so much demand for knowledge. There is every indication that this demand will continue to increase, and that it cannot be fulfilled by conventional methods.

H. Kay

BREATHING

Under the chairmanship of the Section's president, Sir Bryan Matthews, a symposium on "Breathing (External Respiration)" was held on September 4 by Section I (Physiology and Biochemistry) during the recent British Association meeting in Norwich. Four papers were presented, covering varied topics connected with respiratory physiology. Two of these had a clinical bias, and concerned the distribution of ventilation and flow of blood in the lungs, and the effects of oxygen on breathlessness. The other two demonstrated the application of respiratory physiology to aviation, one concerning the design of an aircraft breathing system, and the other describing the results of research into the effects of positive acceleration on the mechanics of respiration.

The symposium was opened with the paper "Variation of Ventilation and Blood-Flow in Different Parts of the Lungs in Health and Disease", presented by Dr. P. Hugh-Jones, physician at the Hammersmith and King's College Hospitals. Starting by showing a model of the air passages in the lungs, and demonstrating how a two-lumen tube could be used to collect expired air from the two lungs separately, Dr. Hugh-Jones went on to describe the limitations of X-rays as aids to studying lung function. Chest radiographs were shown to illustrate this, where there was a complete dissociation between the X-ray appearance and the subject's exercise tolerance. Two new techniques were then described which could give details of local lung ventilation and blood supply, full tribute being paid to the advances of the physicists which had made them possible. In the first, the subject is merely required to take in a quick breath of air containing a minute trace of radioactive oxygen (oxygen-15), and then to hold his breath for about 30 sec. Counters arranged to look at different areas of lung tissue through the chest wall sense the arrival of the oxygen-15 in their fields and then follow its disappearance as it is washed away in the pulmonary circulation. The rate of rise of the counting-rate is a measure of ventilation, and the rate of the ensuing fall in counting-rate is a measure of the local blood-flow. The 2-min. half-life of oxygen-15 is a disadvantage of the technique, but was immaterial at Hammersmith, where it is prepared on the spot in the Medical Research Council's cyclotron. Other gases with longer half-lives might be used elsewhere where such facilities did not exist, for example, radioactive isotopes of argon or krypton. Carbon dioxide made from oxygen-15 had been found to give better results when the technique was used for studying pulmonary blood-flow.

The second technique described by Dr. Hugh-Jones was that of regional gas analysis, using a mass spectrometer which could provide almost continuous analysis of up to four gases simultaneously. Samples were taken through a 0·5-mm. diameter tube which could be inserted into any chosen part of the lung during bronchoscopy. This allowed a study to be made of ventilation and blood-flow in any small area of the lungs, and information obtained in this way would be of value not only to the experimental physiologist but also to the physician and surgeon.

The second paper, "Physiological Requirements in the Design of Aircraft Breathing Systems", was presented by Squadron-Leader J. Ernsting of the Royal Air Force Institute of Aviation Medicine. The variation in properties of the atmosphere with increasing altitude was described, and it was shown that the two main problems to be overcome at a flight-level of 40,000 ft. were an air temperature of − 55° C., and an air pressure of 1/5 of an atmosphere. Ideally, aircrew and passengers should be provided with sea-level cabin conditions at any aircraft altitude, but the weight penalty of the structure needed to withstand the required pressure differential is too great, so that the physiologists reach a compromise with the engineers, and a cabin pressure equivalent to an altitude of 8,000 ft. is chosen. Though this is acceptable, the possibility of a failure in the pressurization system must be allowed for, and with this in view the physiological effects of decompression had been studied. In a civil air-liner, window or valve failure will result in slow loss of pressure over several seconds. In military aircraft, on the other hand, a smaller cabin, together with the necessary provision of aircrew escape systems, makes an explosive decompression, where ambient pressure is reached in 1/10 sec. or less, a real possibility. Dr. Ernsting went on to describe the effects of explosive decompression, first, those due to the low final pressure, anoxia and decompression sickness, and secondly, those due to the rapid change in pressure. Protection against anoxia could be provided by the breathing of oxygen between 10,000 ft. and 40,000 ft., and the breathing of oxygen under pressure at higher altitudes. Above 50,000 ft., the pressure required is so great that counter pressure has to be applied to the rest of the body to make breathing possible, and since at 63,000 ft., the vapour pressure of water at body temperature is equal to the barometric pressure, some form of pressure suit is required to prevent vaporization of body fluids. Experiments on aircrew were described with a short film in which explosive decompression from 8,000 to 38,000 ft. was followed by short periods of impaired function, even when the decompression was followed by breathing 100 per cent oxygen within 5 sec. It had been found that the subject must already be breathing at least 40 per cent oxygen at the time of decompression if impairment of function were to be avoided.

Dr. Ernsting concluded by discussing the serious consequences of explosive decompression on enclosed volumes of gas in the body. If the lungs were shut off from the outside air, serious or even fatal lung damage might occur. Masks had to be designed which would allow free escape of air when decompression took place but which would immediately allow oxygen to be breathed under pressure. The sudden expansion of 200 ml. of gas in the gut to 1 litre was less serious but still very uncomfortable.

The third paper, "Breathlessness: its Alleviation by Oxygen in Health and Disease", was presented by Dr. J. E. Cotes of the Medical Research Council Pneumoconiosis Research Unit at Llandough Hospital. Pointing out how appropriately this subject followed the centenary of the birth of J. S. Haldane, he went on to discuss the 12-month mortality figures of a group of 67 men severely disabled with breathlessness, and the curious finding that those whose symptoms were improved on breathing oxygen fared far worse than those on whom oxygen had no effect. If the reason for this were known, then more might possibly be done for these patients.

Dr. Cotes then discussed the development of our knowledge of the effects of oxygen, and showed that as a respiratory stimulant, while lack of oxygen was of less importance than excess of carbon dioxide, it did play a small part at rest, and with increasing exercise it played an increasingly important part. The administration of oxygen improved tolerance of exercise, and prolonged the time for which heavy exercise could be carried out. Oddly enough, 50 per cent oxygen was better than 100 per cent oxygen in this respect, due possibly to the adverse local effects of too high oxygen tensions on the brain, and it was suggested that 50 per cent oxygen might be the better gas for respirators when work has to be carried out in unfavourable environments. A small cylinder of 50 per cent oxygen should allow the wearer to run a mile in 3½ min. A portable respirator was demonstrated, and sets like this were of great value in improving the tolerance of exercise by disabled men, not only subjectively, but also objectively in treadmill tests. The increased work which could be carried out was associated with a reduction in pulmonary

ventilation, the limit to capability of work being reached when the resulting ventilation reached the subject's voluntary maximum.

Dr. Cotes was unable to say why the breathing of extra oxygen should produce these effects, but many possibilities had been excluded, such as secondary changes in the pulmonary circulation, blood lactate-levels, or in the stiffness of the lungs. This was the limit of present knowledge on the subject, but it was hoped that the mechanisms operating would soon be elucidated.

The fourth and final paper in the symposium was given by Flight-Lieutenant D. H. Glaister, also of the Royal Air Force Institute of Aviation Medicine, under the title "Some Effects of Positive Acceleration on Respiratory Mechanics". Describing the interest physiologists have had in the effects of posture on breathing, Dr. Glaister went on to show how the human centrifuge could be used to extend data beyond the 1*g* which normally limited laboratory experiments. Positive acceleration was described as that acting in the same sense as gravity on a man standing on Earth's surface, and is measured in terms of this being 1*g*. The postural change from lying to standing was thus equivalent to a change from 1*g* transverse acceleration to 1*g* positive acceleration.

Circulatory effects prevented many experiments being carried out above 4 or 5*g*, since the degree of relaxation required to produce accurate respiratory measurements resulted in blackout or even unconsciousness. Most comparisons were therefore made between 1 and 3*g*. As positive acceleration was applied the diaphragm was pulled down by the weight of the abdominal contents, and at 3*g*, about 500 ml. of air were drawn into the lungs. Despite this, the vital capacity of the lungs fell by 300 ml., due partly to an increase in stiffness of the chest wall, and partly to a rise in intra-abdominal pressure which limited the full descent of the diaphragm. The basic mechanics of respiration were then described, and it was shown how the various properties of lung and chest wall could be isolated and measured. Results of experiments using these techniques had shown that no change occurred in the stiffness of the lung, nor in the air resistance of the passages leading to it, at least when the subjects were submitted to positive accelerations of 3*g*.

The work done on the lungs had been measured by recording continuously the changes in their volume and the pressure gradient across them during quiet breathing. This was shown to be unchanged at least up to 3*g*. The total work done by the muscles of respiration, however, had to rise under the effects of increased positive acceleration, due to the stiffening of the chest wall and to the raised intra-abdominal pressure. The overall efficiency of the respiratory process thus fell during positive acceleration. Dr. Glaister concluded by pointing out that while this lowered efficiency was probably of little moment at the lower *g*-levels, and with a subject at rest, it would impose a marked limitation to tolerance of exercise during prolonged positive acceleration.

The symposium concluded with a brief discussion on the four papers. D. H. Glaister

THE NORMAN LOCKYER OBSERVATORY

By Prof. G. K. T. Conn
Department of Physics, University of Exeter

Sir Norman Lockyer founded and endowed the Hill Observatory at Salcombe Regis, Sidmouth. He was its first director. It is now the Norman Lockyer Observatory and has been, since 1948, intimately associated with the University of Exeter. Lockyer was a remarkable man. His discovery of helium in the Sun's spectrum in 1868 was made as a "private citizen", for he was not appointed to the Science and Art Department at South Kensington by Disraeli until 1875. On the formation of the Royal College of Science he became director of the Solar Physics Laboratory and professor of astrophysics. When the Solar Physics Laboratory at South Kensington was transferred to Cambridge in 1913 he set about building a new observatory on the superb site at Sidmouth, at the age of seventy-seven. Lockyer was the founder editor of *Nature* in 1869 and remained responsible for fifty years. He was succeeded as chairman of the Observatory Council by Sir Richard Gregory, his successor as editor of *Nature*. Indeed, both Lockyer and Gregory took great interest in the affairs of the old University College of the South West, now the University of Exeter, and it was Gregory's initiative that led to the intimate association of the Observatory with the University. "a possession which is unequalled in any but the older universities".

The peninsula Salcombe Regis is relatively free from industrial haze and Lockyer chose a site with a superb horizon and a very low noise-level; it is now of some 44 acres. The buildings consist of offices and laboratories with a well-equipped library, a store room, a cottage and three domes. The McLean dome houses a 12-in. refracting telescope with an objective prism of 20°, mounted with a 10-in. visual refractor on a Grubb equatorial mounting with electric control. (The donor, Sir Francis McLean, was the co-founder of the Observatory and became known to the public as the first airman to fly under the Thames bridges, in 1912.) The Kensington telescope is a 9-in. refractor with an objective prism of 45°, made by Henri Frères, and giving great transparency in the near ultra-violet, on a Cooke equatorial with Russell control; a 10-in. Cooke visual telescope is mounted alongside. The Mond photographic telescope consists of a four-camera, astrographic equatorial by Cooke with three Zeiss triplet lenses of apertures 17, 14 and 10 cm. and one Zeiss anastigmat, aperture 7 cm. The mounting is designed to allow an uninterrupted circumpolar motion at all declinations; the clock drive is fitted with electric control. There are various pieces of equipment which have not hitherto been set up, for example, a 30-in. reflecting telescope and a 21-in. siderostat.

Work began in 1919, the first paper, published in the *Monthly Notices of the Royal Astronomical Society* in 1920, being "Spectroscopic and Magnitude Observations of Nova Cygni III, 1920". Some 90 research papers have been published, about 40 in the period 1920–36 and 45 during 1936–56, when D. L. Edwards

was director (see p. 112 of this issue). The work has been largely on stellar spectroscopy. Since 1936 the principal lines of work have been the development of methods of measuring stellar gradients and colour temperatures, using the McLean prismatic camera, and a photographic survey of galactic clusters made with the Mond telescope. New ground has been broken in recent years with the initiation of photometric research on the twilight luminescence of atmospheric sodium. The Kensington telescope has been used to study the irregular variable star, γ-Cassiopeiæ; there are some 460 spectrograms of this star in an almost unbroken sequence from 1921 to 1956.

The Observatory was, and is, maintained as a non-profit making corporation through endowments and funds provided by donors. Since 1948 the management has been vested in the Council of the Corporation on which the University of Exeter has a definitive majority. The funds available have never been lavish. Rapidly rising costs since the War, together with the development of sophisticated experimental techniques, have meant that the position of the Observatory as an effective centre of original research has become progressively more difficult and indeed critical. Against the background of developments in satellites and in space research, the extensive recent progress of studies of the upper atmosphere and the revival of interest in astronomy and astrophysics, such a difficult position presents an obvious challenge. The site is large enough for astrophysical work and for an extensive programme of research and teaching in geophysics, a growing interest of the University's Department of Physics, and of geomagnetism, an interest of the Department of Mathematics.

The first immediate change of activity for the Observatory will follow from the interests of Dr. K. Weekes and Mr. A. Nichol. Conditions in the high atmosphere can be studied by various means using both radio and optical techniques. Up to the height of the greatest electron concentration in the F_2 region it is possible, in principle, to infer the distribution of ionization from routine soundings. Above the peak of the F_2 region, artificial satellites may be used, or Thomson scattering of radio waves and optical experiments. An examination of the radio waves emitted by satellites may be used to infer the integrated electron density between the receiver and the satellite. This is relatively insensitive to the distribution of electrons, but as the satellite changes height in the course of a transit, the distribution at satellite height may be inferred. The most valuable direct means of studying the distribution of electrons in the high atmosphere is by the Thomson scattering of radio waves by free electrons. Examination of experiments in the United States to heights of about 700 km. suggests that useful results should be obtained to a height of about 2,000 km. It is commonly believed that the emission lines of OI at 6300 and 6364 Å. are excited by recombination in the F region. The correlation between the optical intensities and radio measurements of ionization clearly must be examined. Moreover, it is possible, with careful techniques, to measure the line-width of the OI lines and so obtain a direct measure of the temperature of the atmosphere at the height of emission of these lines.

To a classical astronomer nowadays anything below a fixed star is no doubt to be termed 'terrestrial', just as to many people any study above roof-level is termed 'astronomical'. The future is seen to lie in the wedding of optical with radio techniques in the fields of astrophysics and geophysics and of geomagnetism. Moreover, the vitality of the Observatory has suffered from its relative isolation. The University did indeed, so Gregory said, acquire "a possession which is unequalled in any but the older universities". The means are there to train students and research workers in the old and in the new techniques of exploring space. The raw material of research is to hand. The Observatory began as Lockyer's hobby in his old age: it has acquired a reputation in astrophysics and an affectionate regard in the south-west of England. By acquiring additional eyes we shall see more and see farther. An old man's hobby has become a young University's opportunity.

LONG-RANGE RADIO COMMUNICATION BY SATELLITE MICROWAVE DIPOLES

AN article under this title detailing the West Ford Project was published in *Nature* of September 23, p. 1237. At the eleventh General Assembly of the International Astronomical Union held in Berkeley, California, on August 24, two resolutions were unanimously adopted concerning that Project and similar space experiments that might have harmful effects on astronomy. At the recent General Assembly of the International Council of Scientific Unions in London, a resolution similar to Resolution No. 1 of the International Astronomical Union was adopted, which refers to the whole of science, rather than to astronomy in particular.

The following is the text of the resolutions adopted by the International Astronomical Union:

Resolution No. 1. *Viewing* with great concern the grave danger that some future space projects might seriously interfere with astronomical observations in the optical as well as in the radio domain,

and *believing* that a degree of contamination of space which at the present time would be hardly detectable, might, if long-lived, well be disastrous to future observations with improved techniques,

and *maintaining* that no group has the right to change the Earth's environment in any significant way without full international study and agreement;

the International Astronomical Union *gives* clear warning of the grave moral and material consequences which could stem from a disregard of the future of astronomical progress,

and *appeals* to all Governments concerned with launching space experiments which could possibly affect astronomical research to consult with the International Astronomical Union before undertaking such experiments and to refrain from launching until it is established beyond doubt that no damage will be done to astronomical research.

Resolution 2. The International Astronomical Union expresses its appreciation that the plans for Project West Ford have been publicly announced well ahead of proposed launching and of the United States Government's official policy* that further

launchings will be guided by the principle that such projects shall not be undertaken unless sufficient safeguards have been obtained against harmful interference with astronomical observations.

Nevertheless the International Astronomical Union views with the utmost concern the possibility that the band of dipoles proposed in Project West Ford might be long-lived, and it is completely opposed to the experiment until the question of permanence is clearly settled in published scientific papers with adequate time being allowed for their study. The International Astronomical Union is opposed to any experiment which might hamper future developments in astronomy.

If a short lifetime for the dipoles and the harmless nature of the experiment can be assured, and if Project West Ford is carried out, the International Astronomical Union regards it as essential that the fullest observations of, and experiments on, the properties and behaviour of the band of dipoles be carried out by all possible means. The observations and experiments should be performed and analysed according to the highest scientific standards and with the best equipment available, bearing in mind that signals which are barely, or not, detectable to-day will probably cause serious interference with future scientific research because of the development of more sensitive equipment.

The observations and experiments to be made on West Ford are likely to be difficult to perform, and will, in many ways, be similar to those carried out by the authorities responsible for operating West Ford. Moreover, much specific information such as precise and up-to-date ephemerides will be required. The International Astronomical Union will attempt to arrange for rapid and full co-operation among astronomers making observations and calculations, and to provide for world-wide dissemination of their results conforming to accepted standards of scientific research.

The International Astronomical Union welcomes the position* taken by the Government of the United States that any decision on later experiments of the West Ford type will be taken in the light of the results obtained from the presently proposed experiment. To enable the International Astronomical Union to obtain the necessary data, it requests the Government of the United States to grant full privileges to a group of astronomers, acceptable both to the Government and to the Union, to co-operate with West Ford authorities in performing quantitative experiments to determine the properties of the proposed belt of dipoles, its changes with time and location, and its impact upon present and future astronomical research.

* Letter of August 11, 1961, from Dr. J. B. Wiesner to Dr. L. V. Berkner.

THE SWEDISH SEED ASSOCIATION

THE title traditionally given in English translations to the Sveriges Utsädesförening does not perhaps fully indicate the scope of the scientific work in this pioneer research station.

Throughout its development the Swedish Seed Association has had strong support from both farmers and scientists, and when the seventy-fifth anniversary of the Association was celebrated in July 1961, King Gustav VI Adolf welcomed a large attendance of members living within reach of each of the eight branch stations as well as growers and technicians who are directly in touch with the work at the Svalöf headquarters.

Founded in 1886 as a co-operative association of farmers on the initiative of Birger Welinder, who farmed at Svalöf, it reflected a big increase in interest in crop-husbandry problems. During the extension of the arable cropping area in the middle of the nineteenth century Swedish farmers had come to realize that the severity of the winter, the relatively long days and the short summers made demands which could not be met by even the best of the foreign varieties.

From the beginning the pioneer plant breeders at Svalöf fought hard to convince the large body of farmers and seed growers of the importance of using the best scientific techniques in the improvement of crops. As the programme developed under Profs. Hj. Nilsson, N. H. Nilsson-Ehle, A. Åkerman and other distinguished scientists and agronomists it became clear that improved crop varieties could best be obtained through an active programme of research some of which must be of a fundamental nature.

Cytogenetic Research

Practical breeding work is undertaken at Svalöf on the herbage crops, oil and fibre crops, and the root crops for fodder production as well as on the four main cereals.

For this breeding work material is provided mainly from three sources :

(1) By the introduction of valuable genes from primitive forms or wild species, or from cultivated varieties with special characteristics. One of the most marked features at Svalöf is the importance attached to collaboration with crop improvement and other research stations in foreign countries. Valuable plant material has been obtained from a wide range of countries either by exchange or by plant collecting expeditions.

(2) By inducing mutations. The Swedish workers are effectively using this technique to increase the genetic variability of the cultivated varieties and breeding material with which they are working.

(3) By increasing the chromosome number through auto- and allo-polyploidy, thus offering new possibilities for the development of new genotypes. Especially promising are the polyploids of red and white clover, rye and turnips. (Oil rape is an important crop in Sweden. By crossing artificially induced polyploids of turnip rape and different types of kale, new high-yielding and hardy varieties of winter rape are being developed.)

Research of a more fundamental nature is in progress to ascertain the causes of reduced fertility in artificial polyploids and to investigate different methods of using polyploids in breeding programmes.

In the development of polyploids the work at Svalöf has benefited greatly from the close co-operation of Prof. A. Müntzing at the Institute of Genetics situated a few miles away in the University of Lund, and similarly with Prof. Å. Gustafsson on induced mutations in barley and other crops. Mutations have been induced in smooth-stalk meadow grass which have brought a return to cross-fertilization as the means of reproduction in place of apomixis.

In this way the range of genetic variation has been greatly increased.

The nature of yield in some species is being investigated genetically and physiologically with the aid of special growth chambers.

Biochemical Investigations

Great importance is attached to the breeding of new varieties of improved quality and in this the chemical laboratory is closely concerned.

More fundamental studies are concerned with the enzyme changes associated with the ripening of seeds. In a country extending as far north as northern Iceland, and as far south as Northumberland, the ripening of grain and other seed crops is of special importance.

Cereal Breeding

The breeding of spring oats at Svalöf has been of inestimable value to Britain.

Victory, Eagle, Star, Sun II and Blenda all came from Svalöf, and these represent some of the best-known varieties grown in Britain during the past fifty years. (A peculiar rhythm of growth is needed in oat varieties grown around Lake Mälaren, where severe drought conditions are likely to occur in spring, and the most successful varieties are those in which the shooting of the tillers is delayed for a period.)

Barley varieties in Sweden cover a wide range of climatic conditions, and Svalöf has been successful in breeding early six-row barleys for middle and northern regions as well as the varieties for southern Sweden, of which Ymer and Freja have been successful in Scotland and England.

Barley has been used as a convenient subject for experiment at Svalöf to find how the mutation process may be directed towards the production of desirable mutants. Much is being learnt by the association of cytogenetical analysis of the mutations with a close observation of field characters in barley.

Herbage Crops

The ecotype concept was largely developed in Sweden, and it is not surprising that special attention has been given to the interesting range of regional types of red clover growing between southern Sweden and the northern limits of the crop. Day-length reactions are being studied in relation to growth rhythm, time of flowering and to the stage at which reserve nutrients are stored. Of notable success is the work on resistance to stem nematodes in red clover.

The Filial or Branch Stations

Most of the new varieties introduced in recent years have been the result of collaboration between the main station at Svalöf and one or more of the eight 'filials' or branch stations. At Ultuna (Uppsala), for example, some of the main work on red clover, lucerne and peas has been undertaken. Vegetable crops, if one excepts the important pulse crop of dried cooking peas, are not at present included in the programme of work of the Association.

Seed Certification and Distribution

To relieve the Swedish Seed Association of the task of multiplying and distributing seed of the Svalöf varieties, the General Swedish Seed Co. was established in 1891. In Sweden, the seed trade is organized differently from Britain, and the Seed Co. at Svalöf finds it necessary to produce large tonnages of seed. This is done partly on the farms owned by the Company.

The Swedish Seed Association co-operates closely with the Central Government Seed Testing Station in the production of State certified seed, and all the seed which leaves Svalöf is of certified grade.

From the beginning the Swedish Government has given monetary support to the plant-breeding work, and this at present amounts to about £135,000 a year. Other sources of income are the profits from the General Swedish Seed Co. at Svalöf, research grants and donations from foundations and private organizations, and fees for analyses made in the laboratories.

Links with Britain

By the year 1919 Svalöf was already well established, and it was natural that representatives of the National Institute of Agricultural Botany, founded in January 1919, should visit Svalöf to take advantage of the unique experiences gained there in crop improvement work. In the party representing the Council of the National Institute of Agricultural Botany was Prof. R. G. Stapledon, then newly appointed to Aberystwyth. The value of the ecological approach to variety testing, and to the collection and use of local varieties and ecotypes developed in Sweden was particularly appreciated by the visitors from Britain. The ecotype concept was to be further developed by Stapledon at the Welsh Plant Breeding Station, and by J. W. Gregor in Scotland.

Exchange visits between Svalöf and the crop improvement stations in Britain have been numerous in recent years during the directorship of Prof. E. Åkerberg, and they have undoubtedly been of great benefit to both countries.

All workers in crop improvement, whether agricultural botanists, plant breeders, farmers or seedsmen, will join in congratulating the Swedish Seed Association on the remarkable achievements of the past seventy-five years and in sending good wishes for the future.

F. R. Horne

OBITUARY

Prof. Eric Bradshaw, M.B.E.

Prof. Eric Bradshaw died suddenly at his home in Marple, Cheshire, on August 15. He was aged fifty-two and was professor of electrical engineering and director of the Electrical Engineering Laboratories in the Faculty of Technology of the University of Manchester at the Manchester College of Science and Technology.

Eric Bradshaw was the son of Lady Bradshaw and the late Sir William Bradshaw, and was educated at King's School, Grantham, and in the Department in which he later became professor. He was a student under Prof. Miles Walker and graduated in the University of Manchester with a degree of B.Sc.(Tech.) with honours in 1930, and with the degree of M.Sc. (Tech.) in the subsequent year. Following his graduation he spent some time with the British Thomson-Houston Company at Rugby.

He became a lecturer and afterwards special lecturer in high-voltage engineering at the Royal Tech-

nical College, Glasgow, during 1933–44. While in Glasgow he was responsible for the inception there of the high-voltage laboratory and gained his Ph.D. at the University of Glasgow. In 1944 Eric Bradshaw moved to his old Department in the College of Technology, Manchester, as senior lecturer in high-voltage engineering; he was appointed professor of electrical engineering in October 1952.

After the War he was a pioneer in the organization of courses at a very high level for the senior engineering staff of the newly nationalized electricity supply industry and was responsible for many such courses at Electricity Hall, Buxton, during the years 1947–52. Most of these courses, changing their content from time to time but retaining Bradshaw's form of organization, eventually became a standard part of the educational activities of the Central Electricity Authority (later the Central Electricity Generating Board) and the Area Boards. During the same period he founded, in 1948, the *Bulletin of Electrical Engineering Education*, and continued thereafter as organizer and editor until his untimely death. This journal to-day circulates to between four and five hundred universities, colleges and other subscribers throughout the world.

Bradshaw's published work and interests lay in four categories. In the field of units, symbols and nomenclature, he was one of the authors of a well-known symposium of papers to the Institution of Electrical Engineers on the M.K.S. system of units, was the author of a book on the same subject, and served on British standards and international electrotechnical committees. During his career at Glasgow and his early years at Manchester he published work on high-voltage measurements using oscillating electrode systems. Immediately afterwards his attention turned to problems of the power supply industry, and he was responsible for several devices for the direct measurement of rate of change of frequency, one of which was published by the Institution of Electrical Engineers. His latest interests lay in the field of electric railway traction. He was responsible for the design and building at Manchester of a track performance calculator for British Railways, the details of which were published in a paper for the Institution of Electrical Engineers; latterly this work had extended to analogue devices for determining short-time thermal ratings of traction equipment.

He was known throughout the length and breadth of Britain for his work in furtherance of higher education; many were the advisory committees, colleges and universities to which he gave his time. Many indeed, furthermore, are the individuals, both within and without his profession, who have reason to be grateful for his kindness of heart. Eric Bradshaw was incessant in his activity on behalf of cases of adversity and hardship which came his way; this particularly, but in no way exclusively, applied to any student who through no fault of his own was likely to be incapable of pursuing his studies.

Eric Bradshaw was a keen amateur musician, played with the orchestra organized from time to time in the College of Science and Technology, and generously gave his time and support to musical events in the College, particularly those centred around the organ. Perhaps his greatest lay interest was in the history of the Industrial Revolution. He was intensely interested in the development of railways and canals, and frequently lectured to societies and schools on these subjects. He married Joyce Ena Smith in 1935, by whom he is survived.

C. Adamson

NEWS and VIEWS

The Norman Lockyer Observatory : Mr. D. R. Barber

Mr. D. R. Barber retired on September 30 from his post as superintendent of the Norman Lockyer Observatory at Sidmouth, after twenty-five years service (see also p. 108 of this issue). He was appointed as assistant to the late W. J. S. Lockyer in September 1936 and on Lockyer's death in December continued with the new director, D. L. Edwards, until 1956, when Edwards died; Barber then became superintendent. Edwards and Barber introduced precise methods of photographic photometry into the evaluation of stellar spectra. From 1938 they applied the Greenwich colour temperature technique in a modified form to the measurement of spectrophotometric gradients and their work on the changes in the continuous spectrum of γ-Cassiopeiæ (*N.L.O. Comm.*, 58, 62, 64) is well known. About this time Barber built the first of a series of night-sky photometers, designed to measure the green, OI, line of the airglow. This instrument was taken to the Lick Observatory, Mount Hamilton, on Barber's appointment to a Martin–Kellogg fellowship in the University of California (1940–41). With this he demonstrated for the first time a definite correlation between the intensity of the green line and geomagnetic activity (*Nature*, 148, 88 (1941)).

During 1941–45, Barber worked at the Kodak Research Laboratories in England, and on his return to the Observatory he became interested in the sodium *D*-line emission in the twilight and night skies, which he studied by photographic and photoelectric methods. He established the presence of highly polarized twilight emission following solar flare and geomagnetic activity (*J. Atmo. Terrestr. Phys.*, 10, 172; 1957), and a dependence of the intensity of emission on lunar phase, presumably because of tidal movement of the upper atmosphere at the level of sodium emission, 70–110 km. (*Intern. Astro. Union Draft. Rep.*, Berkeley Meeting, August 1961; *Rep. Comm.*, 21, *Luminescence du Ciel*). Lately Barber's interests have broadened to include solar–terrestrial relationships; certainly he has already obtained fascinating and remarkable results. Those on the effects of living organisms will certainly keep him occupied in his retirement.

John T. Tate Medal of the American Institute of Physics : Dr. P. Rosbaud

Dr. Paul Rosbaud has been awarded the first John T. Tate International Medal for distinguished service to physics. The Medal is of gold and has been established in order to recognize distinguished service to physics by individuals who are neither residents nor citizens of the United States. 1,000 dollars accompany the award. The late Prof. J. T. Tate, who died on May 27, 1950, was dean of the College of Science, Literature and Arts at the University of Minnesota, and served as research professor of physics,

chairman of the Governing Board of the American Institute of Physics, and managing editor of the American Physical Society.

Dr. Rosbaud before and during the Second World War was science editor for the Springer Company, a Berlin publishing house. As an editor, he is credited with being the driving force behind the creation of new journals and the improvement of existing periodicals, as well as being responsible for the publication of many books by scientists. Born in Graz in 1896, he received his doctorate in Berlin and then carried out research in X-ray crystallography. He joined the Springer publishing house in the 1930's and succeeded in sending its valuable scientific books to safety before the bombing of Berlin. His extraordinary courage and acquaintanceship with friends in high places, who were uncommitted to the Nazi régime, enabled him to keep in touch with scientists in occupied Europe during the War and to boost their *morale*. He had two French war prisoners, who were physicists, staying with him on *parole*, and enabled them to work and keep in touch with scientific developments. They are now prominent atomic scientists.

During the War he risked his life and freedom by communicating with Allied scientists via the Norwegian underground and neutral channels and by being the confidant of German physicists who opposed the decline of science under the Nazis. After the War, he became editor for Butterworth Scientific Publications (London), and later served as scientific director of the Pergamon Press. Since 1956 he has been European editor for Interscience Publishers and a consultant to other publishing companies.

Council for Scientific and Industrial Research: New Appointments

Five new members of the Council for Scientific and Industrial Research were recently appointed, and took office on October 1. They are Mr. L. H. Bedford, director of engineering, Guided Weapons Division, English Electric Aviation, Ltd.; Mr. G. B. R. Feilden, managing director, Hawker Siddeley Brush Turbines, Ltd.; Prof. E. R. H. Jones, Waynflete professor of chemistry, University of Oxford; Prof. O. A. Saunders, professor of mechanical engineering, Imperial College of Science and Technology, London; and Mr. H. C. Tett, chairman and managing director, Esso Petroleum Co., Ltd. Three members who have completed their five-year term of office retired on September 30: the former chairman, Sir Harry Jephcott, chairman of Glaxo Laboratories, Ltd.; Prof. C. E. H. Bawn, professor of inorganic and physical chemistry, University of Liverpool; and Sir Willis Jackson, at present director of research and education, Associated Electrical Industries (Manchester), Ltd. A fourth member, Sir Walter Drummond, who was also due to retire on September 30, has been re-appointed for a period of three months. The present constitution of the Research Council is: *Chairman*, Sir Harold Roxbee Cox; *Members*, L. H. Bedford, Prof. B. Bleaney, Prof. C. F. Carter, Dr. J. W. Cook, Frank Cousins, Sir Walter Drummond, G. B. R. Feilden, Prof. E. R. H. Jones, Vice-Admiral Sir Frank Mason, Prof. O. A. Saunders, Dr. C. J. Smithells, H. C. Tett, Lewis T. Wright; *Secretary*, Sir Harry Melville.

Ergonomics in Industry

Delegates from industry, the trade unions, universities, research associations and Government departments attended the three-day Conference on Ergonomics organized by the Department of Scientific and Industrial Research in London during September 1960. The Proceedings of the Conference have now been published and include all papers and a verbatim report of the delegates' discussions. (*Proceedings of Conference on Ergonomics in Industry, 27–29 September*, 1960, published for the Department of Scientific and Industrial Research by H.M.S.O. Price 12*s.* 6*d.* By post, 13*s.* 4*d.*) Ergonomics, the scientific approach to the problem of fitting the job to the worker, is aimed at improving industrial efficiency by designing equipment so that its operation is within the mental and physical capacities of the average person. The purpose of the conference was to bring ergonomics to the notice of senior people in industry and to provide an opportunity to discuss its wider implications, in particular where ergonomics fits in with the interests of production engineering, equipment design, work study and industrial health.

Mental Health Book Review Index

The *Mental Health Book Review Index*, which is sponsored by the World Federation for Mental Health, the International Council of Psychologists, the American Foundation for Mental Hygiene, and the Research Centre for Mental Health, New York University, lists references to signed book reviews appearing in 150 journals in the English language, of which 133 are still current and publish reviews, regularly or occasionally. The indexing begins with the volumes current in January 1955. Books are listed with references to three or more reviews: when they appear in psychological, psychiatric, or psychoanalytical journals; when the journals represent at least three fields in the behavioural sciences as a whole; when at least three of the journals originate outside the United States, or when the books reviewed are in foreign languages. The bibliography of books in the sciences of human behaviour and the field of mental health which results from this compilation of book reviews is intended for information only, and neither inclusion nor omission of a title implies a definitive selection. In addition to the 2,062 titles listed since the first issue of the *Index*, about 6,900 titles of books with at least one review are on file until they can be listed under these criteria. The *Index* is published annually under the auspices of the American Foundation for Mental Hygiene, the most recent (**6**, No. 11; 1961) being published in July.

Diet of Sheep

Sheep are highly selective in their grazing habits, picking and choosing between the different plants available in a mixed sward, and even between stem and leaf on individual plants. It is only during the past few years that the extent and importance of such sheep preferences in influencing both the diet of the animal and the botanical composition of the pasture have been appreciated. G. W. Arnold, of the Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, has recently described preliminary results of research on the grazing behaviour of merino sheep on the southern tablelands of New South Wales (*Rural Research in the Commonwealth Scientific and Industrial Research Organization*, **36**; June 1961). In one experiment, a seeds mixture of lucerne and *Phalaris tuberosa* was sown in rows and grazed continuously by three-year-old merino wethers. The sheep selected leaf in

preference to stem where this was physically possible. Furthermore, they invariably chose the leaf or stem material containing most nitrogen. In another experiment, a direct relationship was found between the amount of pasture available and the time spent by sheep in grazing. In spite of a progressive increase in the time spent, the sheep were unable to maintain their live-weights as grazing became more time-consuming. The animals grazing longest suffered the least loss of live-weight. This suggests that other sheep stopped eating because of fatigue before their appetites were fully satisfied. Such differences between individual sheep in their capacity to graze for longer periods on poorer pastures could have practical implications, particularly if the character should prove to be heritable.

Geological and Geographical Research in the U.S.S.R.

A review of geochronology research in the Soviet Union is given by D. I. Shcherbakov (*Priroda*, **2**, 9; 1961). The scale appended gives, for example (in million years), Upper Cretaceous 100, Carboniferous 320, Cambrian 570. The oldest Pre-Cambrian 3,400–3,500. A. I. Perel'man (*Priroda*, **1**, 41; 1961) is continuing his discussion of geochemical landscapes by applying geochemical studies to the previous periods of the Earth's history, thus contributing to a new science, named by A. A. Saukov "historical geochemistry". R. V. Teis (*Priroda*, **4**, 31; 1961) provides a short summary of the work on oxygen isotopes in the Soviet Union, especially of the isotope palæothermometry as applied to the calculation of the temperature of ancient seas as determined by the date derived from sedimentary rocks of the Russian platform and those of the Crimea. Isotope methods were also used to determine the geochemical history of atmospheric oxygen especially during the process of photosynthesis. The Lake Balkhash, in spite of being situated in a semi-desert zone and prone to evaporation, has a very low salinity, namely, 2·84 gm./l. According to M. N. Tarasov (*Priroda*, **2**, 72; 1961) this may be due to the infiltration or seepage of the Lake waters into the fringing rocks surrounding the numerous bays of this Lake. Other suggestions and hypotheses are also discussed.

Angara–Yenisei Hydroelectric Schemes

A short review of these schemes has recently been prepared by P. M. Dmitrievsky (*Priroda*, **1**, 25; 1961). Both the Rivers Angara and Yenisei will have 'cascades' of six dams, providing power for hydro-electric stations. The largest of these, situated at Bratsk, on the River Angara, was begun in 1955. Its head is more than 100 m.; power, 4·5 million kW.; and annual output, 21·7 milliards kW./hr. The annual output of the Angara set will be 70 milliards kW./hr., and of the Yenisei scheme 120 milliards kW./hr. Completion of some units is expected by 1965. The area served by these two schemes is rich in coal, iron ore, aluminium ore and salt.

High-Temperature Induction Furnaces

The design of laboratory high-temperature induction furnaces has been examined in both theoretical and practical aspects by P. H. Dundas, of the Carbon Research Laboratory, Chemical Engineering Department, Imperial College of Science and Technology, London. Dundas has attempted to simplify an otherwise complex electrical engineering analysis, so that the research physicist can avoid wasting much time and energy over trial and error methods when faced with problems of high temperature that can be satisfied only by the use of induction heating. An article in *Research Applied in Industry*, published by Butterworths, 88 Kingsway, London, W.C.2, provides a new approach to the use of induction heating as an important high-temperature tool (**14**, No. 7; July 1961).

Histoplasmosis

The discovery of benign histoplasmosis in 1945 spurred interest in a disease that had been considered rare and invariably fatal. During the ensuing years, a great deal of basic information has been gathered on the epidemiology of histoplasmosis. Most of this knowledge has been gained through the development and use of immunological tests, case history studies, and the screening of soil samples and animals for the presence of *Histoplasma capsulatum*. In a review of existing information (*Mycopathologia et Mycologia Applicata*, **14**; August 1961), Dr. Libero Ajello, of the Communicable Disease Centre, Mycology Unit, Public Health Service, U.S. Department of Health, Education and Welfare, describes how the culture of internal organs of domestic and wild animals has revealed the presence of *H. capsulatum* in different parts of North America, Central America, South America and Africa. It shows also that this fungus apparently cannot multiply in internal organs of birds, the body temperature of which is higher than 40° C. Epidemiological investigations almost invariably revealed that *H. capsulatum* was present in the soil of the region where epidemics occurred. Sometimes the soil containing *H. capsulatum* was transferred by human activities from infected localities through non-infected air, lessening the possibility that the spores of the fungus have been transported by air from the central endemic areas (Ohio–Mississippi–Missouri river valley) to localities far removed from these regions. In the same journal, Henry C. Sweany considers the diagnosis of histoplasmosis and shows that the disease has such a clinical variability that it may simulate not only tuberculosis, sarcoidosis, lymphomas and certain severe anæmias, but it may also occur together with any one of them, which makes the diagnosis all the more difficult. In 25 per cent of the cases studied, it was associated with tuberculosis.

The Australian Mathematical Society

The Australian Mathematical Society met in Brisbane, at the University of Queensland, during May 24–26, and held a joint session with the Australian and New Zealand Association for the Advancement of Science on May 29, 1961. Sixty members attended. The new Council elected was as follows: *President*, Prof. T. G. Room; *Vice-Presidents*, Prof. A. L. Blakers and Prof. L. S. Goddard; *Publications Secretary*, Dr. G. E. Wall; *Treasurer*, Prof. C. S. Davis; *General Secretary*, Prof. H. O. Lancaster. A successful summer research institute was held at the Australian National University, Canberra, during January and February 1961, with Prof. T. M. Cherry as director. A second institute meeting will be held in Canberra, in January 1962, with Prof. E. S. Barnes as director.

Mathematical Reprints

The reprints of the Chelsea Publishing Co., 50 East Fordham Road, New York, 58, have made available a large number of scarce or unobtainable mathe-

matical classics. The most recent batch illustrates the wide variety which has been selected. Salmon's *Higher Plane Curves* (4.95 dollars) and Bell's revision of Frost's *Curve Tracing* (3.50 dollars) are museum pieces, no longer recommended reading but held in warm affection by all who know them. *Projective Geometry* (4.95 dollars) is a translation of the second part of the 1935 book by Schreier and Sperner, the first part having already been issued in translation by the Chelsea Co., under the title of *Modern Algebra and Matrix Theory*; this completes the version of a work which, if now a little out of date, is still recommended by the experts as a suitable introductory university text on algebra and algebraic geometry. Then an omnibus volume (6 dollars) contains two monographs by Weyl, *Das Kontinuum* and *Mathematische Analyse des Raumproblems*, Landau's tract (1916, second edition 1929) on function theory, which inspired so many undergraduates in the 'twenties, and the famous Riemann dissertation, on the foundations of geometry, with Weyl's notes. The books are well produced and stoutly bound, and many libraries, private and public, may be glad of the opportunity to fill gaps reasonably cheaply.

The International Society for Stereology

THE International Society for Stereology was established during May 1961. Stereology is the science of three-dimensional interpretation of flat images, such as sections and projections, that is, the extrapolation from two- to three-dimensional space. The methods range from the evaluation of shadows through serial sections to statistico-geometrical analysis. Stereology finds applications in microscopical anatomy, electron microscopy, geology, metallurgy, aerial mapping, astronomy and cosmology. The Society is holding its first congress at Key Biscayne, Florida, during April 24–26, 1962. Further information concerning the Society and the congress can be obtained from Dr. Hans Elias, Department of Anatomy, Chicago Medical School, 710 South Wolcott Avenue, Chicago 12, Illinois.

Postgraduate Courses at the Imperial College of Science and Technology

INFORMATION contained in a booklet (*Shorter Postgraduate Courses*) issued by the Imperial College of Science and Technology relates to shorter postgraduate courses to be held at the College during the autumn term of the session 1961–62, and advance information on those to be held during the spring and summer terms. The courses included comprise not only those specially arranged but also a selection of lecture courses forming part of the normal departmental syllabuses, where these are likely to be of interest to students as separate courses and where it is possible to accept part-time students. The booklet is divided into two sections covering part-time courses and short full-time courses. Further information on all these courses can be obtained from the Registrar, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7. Details of full-time postgraduate courses extending over one year are given in a separate booklet, *Postgraduate Courses*, which is obtainable from the Registrar, price 2*s*.

B.B.C. Research Scholarships

EACH year the B.B.C. awards research scholarships (valued at £500 per annum) to university graduates in electrical engineering or physics, to enable them to work at any university in the United Kingdom for a higher degree in those fields of telecommunications or physics which have an application to sound or television broadcasting. This year, three scholarships have been awarded: to Mr. J. Elliott (1 year), of the Imperial College of Science and Technology, London, who has been working under Prof. C. Cherry and Mr. J. Roberts; to Mr. K. L. Hughes (2 years), of the University of Birmingham, for work under Prof. D. G. Tucker and Prof. J. T. Allanson at Birmingham; to Mr. B. J. Tilley (2 years), of the University College of Swansea, who will work at Swansea under Prof. W. Fishwick and Mr. J. MacCormack.

University News: Southampton

THE title of reader in botany has been conferred on Dr. J. M. Lambert.

Loughborough College of Technology

DR. G. F. REYNOLDS has been appointed reader in analytical chemistry at Loughborough. Previously, Dr. Reynolds was a principal scientific officer in the Chemical Inspectorate at the War Office.

Announcements

DR. SIGVARD EKLUND, of Sweden, has been appointed director-general of the International Atomic Energy Agency, in succession to Mr. Sterling Cole, whose term of office expires on November 30.

SIR WILLIS JACKSON has been elected president of the British Association for Commercial and Industrial Education.

STAFFORD BEER, founder of the Department of Operational Research and Cybernetics, United Steel, has now become managing director of SIGMA (Science in General Management), Ltd. SIGMA was recently set up to offer professional services in operational research to industry and Government. Roger Eddison is now director of operations of SIGMA. Previously Mr. Eddison was manager of the Plans and Methods Department in NAAFI.

MR. C. T. MELLING, a deputy chairman of the Electricity Council, has been appointed a member of the Advisory Council on Research and Development of the Ministry of Power, in succession to Sir Josiah Eccles.

MR. L. H. WELCH, recently chief electrical engineer to the London Electricity Board, has been appointed as industrial relations consultant to the British Electrical and Allied Industries Research Association.

SIR CYRIL HINSHELWOOD, past president of the Royal Society, will deliver the 1961 Eddington Memorial Lecture in Cambridge at the Arts School Lecture Theatre, Bene't Street, on November 10, at 5 p.m. The title of the lecture is "The Vision of Nature".

THE Natural Rubber Producers' Research Association is holding a symposium under the general title, "Fundamental and Practical Approaches to Ageing Protection", at its laboratories at Welwyn Garden City during November 2–3. Attendance will be by invitation only. Further information can be obtained from the Natural Rubber Bureau, 19 Buckingham Street, Adelphi, London, W.C.2.

THE Association of Universities of the British Commonwealth has issued invitations to its member universities to appoint delegates to the ninth quinquennial Congress of Universities of the Commonwealth, which will be held at the University of London during July 15–19, 1963, and which will mark the jubilee of the founding of the Association.

COAL SCIENCE

THE biennial International Conferences on Coal Science, which began in The Netherlands in 1955, moved on the most recent occasion to France: the fourth Conference was held at Le Touquet during May 30–June 2. Tradition was followed in the extent of local co-operation, and the Mayor, Dr. Jules Pouget, received delegates and their wives at the Town Hall. A tribute should be paid, for the excellent organization and remarkable hospitality, to the French sponsors, the Centre d'Études et Recherches des Charbonnages de France: particularly to Prof. M. Letort (a director-general of the Centre), who presided, R. Loison, the French member of the International Committee, and A. Ploix, who undertook all the details of organization. The conference was fortunate in gaining Mr. Berkx's experience from the previous meeting which he organized in The Netherlands.

Interest continues to increase; the recent Conference included a delegation from the U.S.S.R., and Spain, Brazil and Czechoslovakia were represented. On the other hand, delegates from India and East Germany, who attended in 1959, were absent. The papers presented originated from ten countries. Titles of more than one hundred were submitted, and this number was only reduced with some difficulty to about 50 by narrowing the field—to papers of direct relevance to the chemistry, physics and carbonization of the coal substance.

Since authors are submitting their papers to journals of their choice and proceedings of the Conference will not be published, the 53 papers presented will all be mentioned briefly, grouped under the three main headings used in the sessions. An independent summary by A. Ladam in the French language will be found in a late-1961 issue of *Chimie et Industrie*. A numbered list of references so far available has been appended.

Chemical Properties

P. H. Given[1] reviewed his proposed molecular model for coals in the light of more recent work on dehydrogenation, nuclear magnetic resonance, and other evidence; he concluded that the general features of the model had been confirmed, but indicated changes in structural detail that appeared desirable.

H. A. G. Chermin attempted to deduce from measured densities and elementary analyses the mean size and proportion of aromatic units in coals, and directed special attention to the accuracy possible. Corrections to previous attempts were made and quite good agreement with X-ray measurements of mean size was found.

H. S. Rao, P. L. Gupta, F. Kaiser and A. Lahiri considered the assignment of the band at 1,600 cm.$^{-1}$ in the spectra of coals. This band could have one or all of several origins; and the results of reducing the coals with lithium aluminium hydride, since this did not cause a change in intensity in the band, were considered to discount assignment to a carbonyl vibration. On a related subject was an investigation, by L. Czerski and L. Czuchajowski, of the same spectral band in polycondensates of *o*-, *m*- and *p*-dihydroxybenzenes with formaldehyde. When the *para*-derivatives were oxidized with ferric sulphate the 1,600 cm.$^{-1}$ band increased in intensity, possibly owing to formation of hydroxyl/carbonyl chelate structures, supporting earlier assignments of this band in coals to such structures.

M. T. Monaghan and K. Littlewood attempted to use trimethyl-acetoaminohydrazide hydrochloride (Girard's reagent *T*) to extract carbonyl-containing substances from coals as water-soluble derivatives (see p. 128 of this issue of *Nature*). M. Weclewska described the effect on eight coals of exposing them to a variety of organic vapours. Conclusions were drawn as to the variation with rank of free and hydrogen-bonded hydroxyl groups. P. H. Given, M. E. Peover and W. F. Wyss had examined the chemical reactions and properties of some petrological constituents of coals. Moderate differences in hydroxyl content and reducibility were found, the general molecular pattern being similar for all the macerals studied. B. K. Mazundar, S. Ganguly, N. Gopal De and A. Lahiri had investigated the effect of phosphoric and sulphuric acids on coal structure; they found that a constant proportion of the total carbon in the coal (92 per cent) was retained in the chars produced when the products were carbonized. Since extensive dehydrogenation did not result, an apparent condensation between the alicyclic structures and the remainder of the molecule was postulated to explain the inhibition of tar formation caused by the treatment. J. D. Brooks and T. M. Spotswood[2] discussed the reaction of carbonized coals with bromine and with sodium in various forms; the results were consistent with the presence of polycyclic hydrocarbon structures.

Several papers were concerned with oxidation or oxidation products. H. E. J. Brusset compared humic acids extracted from low-rank coals with those produced synthetically from polyphenols by oxidation; he adduced support for an analogy between these classes of material. Oxidation of coal in alkaline medium at 260° C. was described by J. E. Germain, J. Guillon and P. Delattre. The high yields of benzene carboxylic acids obtained were extracted with methyl ethyl ketone and subjected to chromatography. Fractions were characterized according to the number of carboxyl groups per benzene ring; increase in rank of coal was accompanied by a decrease in the mean number of groups per ring (about 4) and an increase in total yield of these acids. G. J. Lawson and S. G. Ward reported a further examination of the resolution of sub-humic acids (secondary oxidation products of coal) partly by means of ion-exchange resins. Several known acids were isolated, including some previously reported (for example, glycollic and mellitic acids) and two aliphatic acids containing nitrogen (nitrilotriacetic acid and oxalylglycine). B. Jüttner[3] described the oxidation of coke with nitric acid followed by sodium hypochlorite and extraction with butanone. The yield of mellitic acid increased sharply between 600° and 700° C. carbonization temperature, and then fell slightly. A related topic was a description of the use of ozonolysis in research on coal tar, by P. G. Copeland. Oxidized pitch fractions and model substances were further decomposed with alkaline peroxide and/or permanganate in pyridine and the products were identified. Mechanisms were established from the work with

models, but the products from coal tar pitch had not yet been identified.

Reaction of coals with alkaline solution at 200°–300° C. was found, by M. Ihnatowicz[4], in many cases to increase greatly the agglutinating values observed when the products were later heated. A strong hydrogenating action accompanying hydrolysis was indicated.

Possibly the most novel work described was that of L. A. Herédy and M. B. Neuworth on a depolymerization of bituminous coal at only 100° C. The coal was allowed to react with a labelled phenol–boron trifluoride system; depolymerization was recognized by substantially increased solubility in phenol. Models were used to elucidate the reactions taking place. With coal, cleavage of aromatic-aliphatic linkages and also interchange of aromatic units with phenol were both involved. Some of the structural units were thus characterized in terms of analysis and molecular weight, and evidence of bridges other than *via* methylene groups was obtained; possible model structures for coal that would fit the evidence obtained were discussed. E. E. Donath adduced the presence of polynuclear aromatic units in pitches from coal hydrogenation from a study of the ease of change in hydrogen content.

Two papers considered processes that may occur during the formation of coals. G. Huck, J. Karweil and K. Patteisky[5] showed in an experimental study that only dynamic pressure is likely to accelerate increase in rank, and that spores are unsuitable as indicators of the temperature of formation. I. A. Breger considered the 'coalification' of wood in uranium-bearing sandstones. It seems that nuclear radiation has caused dehydrogenation and condensation, leading to effects similar to those produced by biochemical coalification.

P. Dath, H. Renson and E. H. Grand'ry reported work on the influence of rank and petrological composition on the behaviour of coals in the Audibert-Arnu dilatometer and came to the conclusion that both are important. A. Ihnatowicz, M. Lason and M. Zyla had shown by treatment of coal with alcoholic potash and with pyridine, together with hydroxyl determinations and adsorption measurements, that the two treating agents used altered these properties in a similar manner. Evidence that this was due to a mechanical loosening of the structure was obtained. J. D. Brooks, J. F. Stephens and H. Silberman[6] had examined the X-ray diffraction patterns and functional groups in chars from carbonized salts of brown coal. The 002 band was shifted, and it was suggested that in brown coals this band may possibly arise from an interchain *d*-spacing. Phenolic groups in the salt form were less-easily eliminated by heating than in the untreated coals. B. Roga surveyed Polish and international classifications of coals and suggested modifications.

Physical Properties

S. Ergun and M. Mentser carried out investigations on the X-ray scattering patterns of ten anthracites and *meta*-anthracites. Those of higher rank gave rise to three-dimensional reflexions, indicating that coal can graphitize during metamorphism. Nuclear magnetic resonance techniques were applied by J. Smidt, W. van Raaijen and D. W. van Krevelen to show that the mobility of the protons in coals changes with rank, and that it shows a characteristic variation with temperature for the coking vitrinites. The effect, on earlier published conclusions, of this variation was discussed. Pitches were also studied. M. Jacubowicz and J. Uebersfeld described results obtained with several coals when studied by double magnetic resonance. The nature of the interactions (dipolar, etc.) between the electronic and the nuclear magnetic moments was determined, and a better understanding was achieved of the electron spin resonance ascribed to free radicals. Some possibilities for studying adsorption were also mentioned.

Two papers were concerned with the specific surface and heat of wetting of coals and carbonized coals. S. Prégermain, S. Durif and P. Chiche studied the adsorption of water, methanol and nitrogen on carbonized coals, and their low-angle X-ray scattering. *Inter alia*, a relation was found between the ease of graphitization and the development of the internal surface. L. Robert and S. Prégermain found a quantitative relation between heat of wetting in methanol, per unit surface of the carbonaceous solid, and its oxygen content; extrapolated to zero oxygen content the heat evolved is still considerable, and the chief effect, therefore, is to raise the estimated surface areas of high-rank coals and chars while leaving those of low-rank coals little changed. J. W. Phillips reported on the self-adhesion of coal during briquetting without a binder. The physical properties, and the structure revealed by photomicrographs, of the briquettes were linked with the stress and strain relations during the application of pressure with additional shear strain. D. H. Everett, E. Redman, A. J. Miles and D. H. Davies reported adsorption isotherms on chars made from polyvinylidene chloride. Important differences in hysteresis, relevant to the mechanism of coal carbonization, were discussed.

Reflectances of vitrinites carbonized at a series of pressures were reported by M.-Th. Mackowsky. An attempt was made to relate the changes found with the chemical changes known to occur during heating. L. A. Gilbert had determined refractive indices and absorption coefficients of polished surfaces of coals, in the ultra-violet region, by a polarized light method. This was the first application of this method to coals and yielded valuable results. All the vitrains used showed a strong aromatic-type absorption around 2600 Å. S. Ergun and J. T. McCartney[7] reported the refractive indices of ultra-thin sections of coals and graphite in the visible region and extinction coefficients in the ultra-violet and visible regions. The results were tentatively considered to lead to conclusions regarding the aromatic units in coals similar to those derived from X-ray investigations.

E. de Ruiter, R. Leutner and H. Tschamler reported the electrical resistance of powdered coals under pressure and of their products of extraction by pyridine in relation to temperature. Little change in the energy of electronic activation for coals was observed between 80 and 90 per cent carbon content; values of this energy were similar for the residues of extraction of solvent, but greater for the soluble products by extraction. The results for the extracts were considered to accord with conclusions from X-ray diffraction.

Thermal Decomposition

No less than four papers were concerned with the plastic softening of coking coals in the early stages of heating. Rheological properties during the initial softening had been studied by H. R. Brown and P. L. Waters[8]. They found that the temperatures of

initial softening of a wide range of Australian coals were almost independent of rank within the range of 82–89 per cent carbon content, and that the softening was initially reversible; but maximum fluidity and solidification temperature were dependent on rank. A medium-rank coal behaved as though the flow were Newtonian until the onset of active decomposition. A mean activation energy of about 70 kcal./mole was estimated. J. C. Macrae and K. S. Murthi[9] had determined, in a special cone viscometer, the variations of fluidity at a constant temperature below the active decomposition point; they interpreted these in relation to the microscopically observed porosity of the char after cooling and the amount of chloroform extract obtainable from this char. The duration and magnitude of fluidity were found to be highly dependent on temperature, and the increasing fluidity before the maximum to be directly related to the dilation of the mass with gas bubbles; the amount of chloroform-soluble material present did not change materially over the same period and therefore was considered unlikely to produce this increase.

I. G. C. Dryden and W. K. Joy[10] had investigated the provenance of the fusible chloroform-soluble material in heated coals, by comparing similar materials obtained in three independent ways. They concluded that this fusible material is partly present in the unheated coal and partly formed or disengaged during heating, the proportion originally present increasing with increase of rank up to 100 per cent in prime coking coals. Evidence was also presented leading to the conclusion that the portion originally present is also more resistant than the remainder to thermal decomposition. The plasticity developed was considered, on limited evidence, to depend critically on the concentration of fusible material—not entirely in agreement with the conclusions of Macrae and Murthi. A. D. Dainton demonstrated by static beam-bending methods that visco-elastic behaviour persisted in the semi-coke from a coking coal above the commonly accepted point of solidification for the plastic coal, that is between 480° and 800° C.

Development of the fissures in coke that result from shrinkage, following resolidification of the plastic mass, was studied by H. E. Blayden, H. C. Wilkinson and G. E. Oatley in relation to the temperature distribution in an experimental coke oven. A clear correlation between shrinkage and fissuring was observed, and these authors also concluded that semi-cokes may show visco-elastic behaviour. G. H. Taylor[11] reported the appearance in both reflected and transmitted light of coals at various stages in carbonization. Colour photomicrography was an important feature of the techniques used. Development of an optical mosaic structure from the plastic vitrinite was observed. The behaviour suggested that the resolidification of coke is analogous to a crystallization process. H. Pichler and D. H. Lee[12] reported the effects of extracting a bituminous coal for various times with tetrahydrofuran. The consequent changes in plastic behaviour, dilatometer curves, volatile matter and other characteristics were described. W. F. Landers and J. B. Goodman had investigated the effects of temperature and gas pressure on the tar obtained from a lignite. Increased susceptibility to decomposition of the tar liberated between 500° and 600° C. was evident, and this was further increased by pressure.

S. Černič reported an investigation, with the help of labelled pyrite, of the behaviour of organic and inorganic sulphur-containing constituents of a series of coals during carbonization. Evidence was obtained that inorganic and organic forms of sulphur were interchanged to some extent, according to the rank of coal, during the thermal decomposition. The effectiveness of carbonizing in hydrogen, ammonia and pre-heated steam, in reducing the sulphur content of the resulting coke or char, was also investigated; steam proved the best.

Heats of coking formed the subject of three papers; the definition used must be considered when making comparisons. D. Fitzgerald reported coking heats for an anthracite and several low-rank coals. An isothermal fluid bed calorimeter was used for the measurements. The poor agreement between results of other work was critically discussed. C. Meltzheim, J. Lahouste and A. Boyer had made a direct comparison of two methods of measuring the heats of carbonization, and found them in satisfactory agreement. The net heat of carbonization over the whole range of temperature, for a number of coals, was always exothermic, the value varying with rank in a complex manner in the region of 30–170 (± 40) cal./gm. C. Kröger and J. J. Das[13] had investigated the net heats of carbonization of individual macerals by direct calorimetry. The heats were not directly related to the yields of volatile products. A relation between the decomposition heat up to 500° C. and the hydrogen/oxygen ratio and the carbonyl content of the coals investigated was, however, found. At 1,000° C. vitrinite appeared to give the lowest heat values of the three macerals studied.

The rate of evolution of volatile matter from coal, during carbonization in a fluid bed with sand as heating medium, was interpreted by G. J. Pitt on the assumption that the coal is a complex mixture, each component of which decomposes independently by a first-order reaction. He showed that this assumption can lead to the linear change with logarithm of time observed, and with this assumption deduced from the results a distribution of activation energies (35–75 kcal./mole, maximum at 50–55 kcal./mole).

A continued investigation of the pyrolysis of model compounds believed to resemble coal was reported by D. W. van Krevelen and H. W. den Hartog[14]. Polycondensates in which aromatic and non-aromatic structures (or oxygen atoms) alternated, prepared with labelled formaldehyde, were pyrolysed; from the weight, and analysis of and distribution of radioactivity in the products, conclusions were drawn about the influence of structure on the degree of (temperature) overlap in tar and gas evolution and on the weight loss–temperature curves; and it was concluded that C—O bonds decomposed more readily than C—C bonds.

In the next group of four papers, the work described had been directed towards the establishment of relations between volatile products of the pyrolysis of coal and the chemical structure of the coal itself. G. Claxton described work on the genesis of aromatic hydrocarbons using gas-liquid chromatography. In a coke oven, the peak evolution of non-aromatic hydrocarbons occurred earlier in the heating process than the peak for benzene formation; significant effects of the heating-rate were observed. In laboratory experiments more detailed information was obtained. A. Ladam, R. Ferrand, P. Payen and A. Boyer had studied the constituents of low-temperature tars (550° C.) from vitrinite and exinite concentrates, using vapour phase chromatography. Normal paraffins and olefines arose almost entirely

from exinite, and the results suggested that as much as 10 per cent of the exinite used may consist of chains with about 30 carbon atoms; correspondingly, aromatic compounds from exinite tar were more highly alkyl-substituted than those from vitrinite tar. W. Waddington had compared the relative abundance of isomeric forms in tar with that to be expected from the postulated thermal reactions of a proposed structural model. He suggested that the results obtained support the view that coals contain in part a 6 alkyl-4 propyl-2 methoxy phenol type of structure; this may be gradually converted to other structures by thermal/pressure reactions during coalification. C. Kröger and R. Brücker[13] reported results from high-vacuum pyrolysis of coals and discussed these in relation to the coal structure. Products in the range 300°–575° C. were examined by gas chromatography and other methods. Rate constants and activation energies for the evolution of hydrogen, many simple hydrocarbons and the carbon oxides were estimated: and from these in turn, by means of allocation of reactions to structural elements on certain assumptions, quantitative parameters for 17 elements of structure, such as the proportion of aromatic carbon in the coal, were deduced.

There proved to be a shortage of time for formal discussion, and on the other hand many delegates remarked on the value of spontaneous extra-mural discussions. Further experiment is clearly necessary in an attempt to correct this lack of balance. It is hoped that, since these conferences continue to produce more novel material than the average delegate can comfortably digest, further innovations will be made at the next conference, to be held at Cheltenham during 1963. I. G. C. DRYDEN

[1] Given, P. H., *Fuel*, **40**, 427 (1961).
[2] Brooks, J. D., and Spotswood, T. M., *Proc. Fifth Bien. Carb. Conf. U.S.A.* (1961).
[3] Jüttner, B., *Brennstoff-Chem.* (in the press).
[4] Ihnatowicz, M., *Prace Głównego Instytutu Górnictwa* (in the press).
[5] Huck, G., Karweil, J., and Patteisky, K., *Brennstoff-Chem.* (in the press); *Freiberger Forschungshefte, A*.229 (1961).
[6] Brooks, J. D., Stephens, J. F., and Silberman, H., *Proc. Fifth Bien. Carb. Conf., U.S.A.* (1961).
[7] Ergun, S., and McCartney, J. T., *Fuel*, **40**, 109 (1961).
[8] Brown, H. R., and Waters, P. L., *Fuel* (in the press).
[9] Macrae, J. C., and Murthi, K. S., *Fuel* (in the press).
[10] Dryden, I. G. C., and Joy, W. K., *Fuel* (in the press).
[11] Taylor, G. H., *Fuel* (in the press).
[12] Pichler, H., and Lee, D. H., *Brennstoff-Chem.*, **42**, 241 (1961).
[13] Kröger, C., and Das, J. J., *Brennstoff-Chem.*, **42**, 223 (1961).
[14] Van Krevelen, D. W., and den Hartog, H. W., *Brennstoff-Chem.* (in the press).
[15] Kröger, C., and Brücker, R., *Brennstoff-Chem.*, **42**, 245 (1961).

BIOCHEMICAL ENGINEERING

A SYMPOSIUM on "Biochemical Engineering" was convened by the Institution of Chemical Engineers on May 30; it is doubtful whether any two of the participants, lucid as they were on their own topics, would have agreed on a definition of the title. Though, on one hand, one may assume unanimity of outlook in chemical engineers, the term 'biochemist' has been used by many disciplines, from the quantitative biologist to the organic chemist by way of the medical analyst. Moreover, in the fields where engineers encounter Nature in the raw, ought not such a title to be amplified to 'biological chemical engineering'? It is easy to see how confusing such exercises in labelling can be, though there is always a stimulation of interest and research by collecting certain topics together under a high-sounding title, however arbitrary.

Nevertheless, many industries are now based on the controlled use of biological processes. (Strangely enough, the largest industry of this type, brewing, was already developed more or less to its present size before the terms 'biochemist' or 'chemical engineer' existed.) It was the production of antibiotics by fermentation, especially under the high pressure of the Second World War, that brought a variety of biological interests (botanists, zoologists, pharmacists, agriculturists, entomologists—for there were very few true biochemists, by any definition, available at the time) face to face with the engineer (rarely of the 'chemical' variety). The difficulties of communication were considerable and were overcome the hard way. It was also realized that similar problems existed in the food preservation industries and the sewage and effluent disposal services. To help with these problems, the establishment of courses in 'biochemical engineering' were proposed—seldom, however, with any clear idea of what the graduates should do afterwards, although the vague idea of a superman who could control both sides of the industrial scene may have been in the minds of some proponents. The problems were considerable, for whereas most of the engineers' deficiencies were conceptual and philosophical, most of the biologists' were factual and mathematical. Making good the former must have seemed easier or, perhaps, they were not so apparent and the new discipline became, at least for a time, the protégé of chemical engineering.

Two difficulties then faced the new dispensation: first, that with normal chemical engineers at a premium, the supply of new graduate output could not hope for many years to affect the position; second, that with the enormous rate of growth of each parent topic, no person could hope to keep up to date in both biochemistry and chemical engineering. It was clear, therefore, that for many years to come the two functions of engineer and biologist would have to be realized by specialists in each discipline working side by side as separate entities. It was with this aim of bringing together the two groups that this symposium was called.

In the opening paper, Dr. D. J. D. Hockenhull reviewed the formal aspects of the production of economically desirable products from micro-organisms. He stated, as an axiom, that the primary function of a living organism is to reproduce itself. It does this by a complex interlinked system of biochemical reactions on the nutrients supplied to it. Interference with these processes may lead to the accumulation of intermediates or of alternative products derived from them. Industrially important examples of such interference were: unbalanced nutrition (acetic acid), deficient aeration (alcohol), mineral deficiency (citric acid), use of trapping agent (glycerol) or of foreign precursors (penicillin) or inborn error of metabolism (lysine). It was pointed out that these 'primary colours' of metabolic interference, in Dr. G. L. Solomon's words, were most usually applied in combination to-day to obtain the best results. A more

detailed account of the biosynthesis of penicillin was then used to illustrate the concept of controlled fermentation. In the early days, it used to be sufficient to choose an appropriate medium, inoculate it with the organism and leave the fermentation to proceed under set conditions until the time for collecting. To-day, it has been found that strict control of *p*H value, sugar-level and the rate of supply of nutrient are all essential for maximal yield. Such control brings fresh problems for the engineer in the design of suitable equipment. Sensing devices capable of withstanding the conditions of sterilization and of operating satisfactorily in fermentation broths are needed. It is particularly important in this context that the biochemist and the engineer should have a true appreciation of each other's problems.

The engineering problems associated with large-scale fermentations are mainly those of designing a bacterium-tight fermenter and supplying it with large quantities of sterile air. Then there are the associated problems of finding the best methods of aerating and agitating the fermentation broth to promote maximum yields of antibiotic and elimination of the foam that frequently occurs.

Other papers were concerned with more specific problems. The highly aerobic fermentations, as used for production of penicillin and streptomycin, present their own problems of aeration and agitation. Not only must the air supply be sufficient to provide all the oxygen the organism requires but it must also strip the fermentation broth of carbon dioxide which becomes toxic if allowed to build up to a high concentration. Agitation is necessary, first, to break the air into small bubbles, thus presenting a large surface area to the broth, and, second, to provide the shear action that aids mass transfer. Other functions of agitation are to keep solids and mycelium in suspension to ensure that the fermentation broth is of uniform composition. Good mixing is also important for close control of temperature. Efficient agitation also breaks down the mycelium and prevents it growing into large aggregates or clumps.

Two papers were presented on the separate aspects of aeration and of agitation of fermenter broths. Prof. P. H. Calderbank, in his third paper on physical rate processes in industrial fermentations, examined the rate of mass transfer from the liquid to solid particles suspended in mixing vessels. An attempt was made to simulate a mycelial suspension by the use of screened ion-exchange resin particles. The rate of mass transfer was assessed by introducing the acid form of the resin into a dilute sodium hydroxide solution and noting its rate of change of *p*H with time under the desired conditions of agitation and aeration. A serious restriction in transfer occurred if the solid were allowed to aggregate into large clumps. The process of liquid-to-solid mass transfer seemed to be rate-determining in many industrial fermenters.

Dr. N. Blakebrough considered aeration and agitation from a different point of view, using a photographic technique developed to examine patterns of flow in a mixing vessel. The importance of work on flow-patterns, for large-scale operations, is not so much to find out the actual distribution of velocity but rather to confirm that the broth in all parts of the fermenter is being agitated so that no stagnant zones exist.

Both Prof. Calderbank and Dr. Blakebrough approached their problems as chemical engineers. They had used bench-scale apparatus with low-viscosity Newtonian liquids, very different from the viscous non-Newtonian broths often used in industrial fermentation practice and requiring agitation and aeration in vessels of, say, 10,000 gal. capacity. It is to be hoped that, in future, more work will be directed towards the study of non-Newtonian liquids, and that some indications will be obtained on the methods of scale-up needed for predicting with confidence the degree of mixing in a large fermenter.

In aerobic fermentation of pure cultures, sterility is a special problem as the volumes of air involved are large. For example, a fermenter of, say, 10,000 gal. capacity could be fed with 1,000 cu. ft./min. air. In industrial practice absolutely sterile air can rarely be obtained. A compromise is made between the effectiveness of the sterilizing treatment and the detrimental consequences of an infection. For design purposes, a penetration of 1 in 10^{12-13} has been suggested ; that is to say, out of every 10^{12-13} organisms in the air presented to the sterilizing device only one should pass through into the fermenter.

In laboratory-scale work the most common way of sterilizing air is to pass it through a cotton-wool plug or an absorbent filter. On the large scale, filters of glass wool, slag wool or powdered carbon are generally used. Some form of air heater may also be used. The main interest in Mr. R. Elsworth's paper lay in the demonstration that sterilization by heat to kill infecting organisms can be considered as a simple chemical reaction the rate and extent of which can be predicted from an equation of the Arrhenius type. When absolute sterility is not essential, the information presented in this paper can be most useful for determining the time/temperature relationship necessary for a desired penetration.

In the laboratory, experimental work to improve the performance of a fermentation is normally carredi out with shaken conical flasks ; this is the cheapest way of finding out the effects of variations in the strain of organism or the medium used to grow it, for replicate cultures are easy to set up. However, the characteristics of aeration and agitation in shaken flasks are very different from those in conventional deep fermenters. Intermediate development work is therefore also carried out in bench-scale fermenters having a capacity of about 1 gal., and in pilot-plant units of several hundred gallons capacity.

Dr. A. Parker presented a paper on equipment for such laboratory and pilot-plant scale fermentations. After a brief review of the literature of this subject, he divided his paper into two parts, the first dealing with laboratory-scale vessels of 5-l. capacity and the second with 220-gal. pilot-plant fermenters. The wide diversity of engineering problems associated with the design of fermenters clearly illustrated the relationship between the engineering designer and the biochemist using his equipment. Another point made was the great similarity in fermenter design regardless of its size. Particular attention was needed in the design of agitation-shaft glands, the techniques of aseptic sampling and the transfer of seed cultures. Rates of oxygen transfer, as measured by the sulphite method, were given for the fermenters described, under various conditions of agitation and air flow. Sulphite oxidation-rates could not necessarily be correlated with actual fermentation results, but they did serve as a useful index of performance and were also helpful in comparing different agitation systems.

Aeration processes for the biological oxidation of waste waters were described by Mr. A. L. Downing. In effect, the activated sludge process is a very dilute fermentation. The whole process must be carried out as cheaply as possible. Various devices used for aerating the waste liquors were described, such as the induction of air through a Venturi tube and the use of a submerged impeller and sparger. The effects of bubble diameter and depth of liquid through which the bubbles travel were also studied in tap water containing 5 p.p.m. anionic surface-active agent. The effects of surface-active agents on mass transfer were found to depend on the method of aeration. As would be expected, the rate of absorption was roughly inversely proportional to the bubble diameter, and the oxygen absorbed per unit consumption of energy tended to decrease with increased depth of liquid.

The shortness of the Symposium imposed very strict scheduling and handicapped free interchange of opinions. The discussion was held back until several principal papers had been given, and this led to some 'cooling off', which was also encouraged by the separation of question and response. There was a tendency for many of the contributions to be too restricted in scope and lacking in general philosophical content. Perhaps this is a way of saying that they were too specialized for one-half of the audience. Again, a contribution on equipment design, or even some account of the problems associated with it, even to such special details as design of valves, sampling devices, probes and electrodes, might have brought a little more practicality and scope for argument.

The meeting, although demonstrating clearly that biologists and chemical engineers speak rather different languages even when working on the same processes, did indicate that the barriers are not impassable. Moreover, who knows, in crossing the perilous mountains between the two disciplines, one might come across that 'abominable noman', the biochemical engineer. The proceedings of the symposium are to be published in *Transactions of the Institution of Chemical Engineers*. D. J. D. HOCKENHULL

COSMIC RAY RESEARCH IN LATIN AMERICA

THE third Latin-American conference and course on cosmic ray physics was held in Mexico during June 26–July 5. It was arranged by Profs. M. S. Vallarta and J. Roederer on behalf of the Latin-American Council for Cosmic Ray Research under the auspices of Unesco, the Mexican Nuclear Energy Commission, and the U.S. Space Science Board.

The meetings were held at the University of Mexico, famous for the elegance of its remarkable architecture, and at the headquarters of the Nuclear Energy Commission. There were present some 25–30 representatives from Argentina, Bolivia, Brazil, Chile, Mexico, Peru, the United States and the United Kingdom. The course consisted partly of original contributions and partly of lectures of a review nature. The topics covered in the lectures included the nature and origin of the galactic cosmic rays and solar-produced high-energy particles, the intensity variations of the galactic radiation and their relation to solar activity and the structure of the interplanetary magnetic field, the motion of primary cosmic rays in the geomagnetic field, the properties and origin of the Earth's radiation belts, and solar radio astronomy.

The Latin-American countries between them operate a chain of neutron monitors covering a wide belt of latitude from Mexico in the north to Ellsworth in Antarctica, and a good deal of the original work reported at the meeting was concerned with the analysis and interpretation of the data from these stations.

In particular, the remarkable cosmic ray intensity variations during July 1959 and November 1960 (when there were examples both of decreases in the intensity of galactic particles and of injection of additional low-energy particles by the Sun) have been very carefully analysed by the Argentinian, Bolivian and Brazilian groups. In discussing the events of November 1960 the Argentinian workers arrive at the conclusion that the main intensity increase starting at 1930 U.T. on November 12 was due to the envelopment of Earth by a cloud of magnetized solar plasma containing trapped solar particles generated by the 3+ flare which began at about 1330 U.T. on that day. They also conclude that the presence of the magnetic field associated with the plasma was responsible for producing the decrease in intensity of the galactic cosmic rays observed at this time. They attribute the increase in intensity of solar particles between 1330 and 1930 U.T., prior to the main increase, to leakage from the trapping field in the plasma.

The Bolivian group, which operates the extremely well-appointed laboratory at Chacaltaya (altitude 5,200 metres above sea-level) includes in its cosmic ray activities the study of both large air showers and intensity variations related to solar processes. Included in the work reported by this group was an account of the rapid, short-lived intensity decrease observed on November 13, 1960, by means of its neutron monitor, which has the very high counting-rate of $\sim 5 \times 10^5$ per hr. Immediately after the magnetic storm sudden commencement at 10 hr. 21 min. U.T. on that day, the cosmic-ray intensity started to decrease and shortly after 1300 hr. U.T. it dropped suddenly, remaining 3 or 4 per cent below the general prevailing level for about 1½ hr., after which it returned suddenly to the pre-drop value. Analysis of data from other stations showed that this phenomenon happened on a world-wide scale, and the Bolivian group concludes that this kind of modulation of the galactic cosmic-ray intensity could only be produced if the solar plasma contained a very well-defined region of relatively high magnetic field strength.

The work of the cosmic ray group in Santiago at the University of Chile, reported by Prof. G. Alvial, is principally concerned with the investigation of the primary cosmic rays using nuclear emulsion techniques, and he described work which has been carried out on charge determination of heavy nuclei in a stack of *G*-5 emulsion exposed at 131,000 ft. over Minnesota. Charge determination by two methods

gives agreement to better than one unit. One particle has been found with $Z = 30$.

Mrs. R. Gall, of the Instituto of Geophysics, University of Mexico, gave an account of the work carried out there by the theoretical group on the trapping of charged particles in the geomagnetic field and on the relation between the radiation belts and the inner allowed region of Störmer. Work is also being carried out on the motion of cosmic ray albedo particles in Earth's field and on the changes in threshold momenta resulting from the secular variation in the geomagnetic field. At some points on Earth's surface these changes have amounted to as much as 25 per cent during the past hundred years.

This course, which is a biennial event, provides, in addition to the presentation of original work and review lectures, a forum for the discussion and co-ordination of future programmes in cosmic-ray research in the participant countries. The two previous courses were held in La Paz, Bolivia, and San Carlos de Bariloche, Argentina, and it is intended that the next one should be held once again in Bolivia. In a report of this length it has only been possible to select a few representative aspects of the work reported by the Latin-American groups. H. Elliot

PHARMACEUTICAL CONTROL IN BRITAIN

In his chairman's address to the British Pharmaceutical Conference, held at Portsmouth during September 18–22, Dr. D. C. Garratt states a case for a more effective control of the quality of medicinal substances. Drugs not governed by official standards should not become available to the public except under a notification scheme, similar to that already in force for pesticides, so that their clinical characters and standards of purity, with appropriate methods of analysis, would have prior approval. Standards for all medicines should be enforced by testing in regional laboratories specialized for the purpose and maintained by the Ministry of Health. For reputable approved firms, the contractor's own inspection organization could be given the initial responsibility for compliance of goods with specification. The pharmaceutical analysis involved should be admitted as a distinct discipline, by the institution of special training courses and recognized qualifications.

At present, control analysis is too often rendered pointless by insufficient attention to the precision of the methods involved; and for a full realization of the potentialities of such techniques as, for example, ultra-violet spectrophotometry, a national collection of standard specimens of authentic materials of known impurity content is essential. Authority for the enforcement of those standards should be incorporated in an Act of Parliament.

It should be every analyst's concern to consider what can be done to reduce costs of examinations, for example, by using equally effective, but cheaper, solvents, by applying rapid instrumental techniques or, where possible, by replacing costly bio-assay investigations by simpler paper chromatographic techniques.

Dr. Garrett suggests that the public would receive better protection if the control of drugs were divorced from the present Food and Drugs Act and incorporated in a new Medical Substances and Preparations Act built around Sections 11, 12 and 13 of the Pharmacy and Medicines Act. Imported drugs should be subject to stringent examination by analysts of acceptable status. Such status might be conditional on the possession of a special diploma incorporated as a requirement of competence in the proposed Medical Substances and Preparations Act. Examinations for such diploma qualifications would be conducted by those chemical or pharmaceutical institutions approved by the Ministry of Health.

THE LABORATORY OF THE GOVERNMENT CHEMIST

The very varied work done in the Laboratory of the Government Chemist during 1960 has recently been reported*. The Laboratory, which forms part of the Department of Scientific and Industrial Research, exists to provide a service to any Government department that requests it on matters broadly concerned with chemistry, and more particularly with chemical analysis. The Laboratory is the oldest Government chemical institution in the Commonwealth. It was created in 1842 by the Board of Inland Revenue, principally to assist the revenue authorities in detecting and preventing the adulteration of tobacco with worthless additives. The special responsibilities which the Laboratory retains to H.M. Customs and Excise are highlighted by the number of samples received from this authority. Of a total of 327,460 samples examined and reported on by the Laboratory during the year, no less than 280,125 were submitted by H.M. Customs and Excise. These samples cover a wide range of dutiable commodities such as wines, beers and spirits, sugar, textiles, fuel oils, etc.

The statutory duties of the Government Chemist under the Food and Drugs Act, the Fertiliser and Feeding Stuffs Act, and the National Health Act, involve the Laboratory in such work as the analysis of orange juice, dried milk, fertilizers, pesticide chemicals and formulations thereof, pharmaceuticals and drugs, canned foods. Examples of the variety of this work are the determination of glycogen in oysters and the iodine content of school dinners.

An organization which exists to provide an advisory service must carry out a considerable number of routine analyses as, for example, on the variety of materials purchased by various Government departments ; this work varies little from year to year.

Important work from the public health point of view concerns the examination of water and sewage. Much attention is given to the control of fluoridation in municipal supplies (where this is practised). The control of micro-organisms in the Serpentine bathing area is an item of special interest to Londoners.

* Department of Scientific and Industrial Research: Laboratory of the Government Chemist. Report of the Government Chemist, 1960. Pp. iv + 79. (London: H.M.S.O., 1961.) 5s. net.

The Laboratory of the Government Chemist is very often involved in legal cases arising out of such illegalities as the dilution of beer, illicit distilling and forgery. The present report gives some excellent illustrations on the methods used to detect forgeries.

Although mainly concerned with the utilization of existing analytical methods, the Laboratory carries out a considerable amount of research work on the improvement of analytical methods and their adaptation to meet problems peculiar to the types of sample received for analysis. The report also indicates that the small but increasing research programme on the development of new methods of analysis is meeting with considerable success. The new radiochemical section has begun work for municipal water supplies, and on the analysis of effluents from atomic energy establishments.

This report shows admirably that as a means for safeguarding the public interest over a wide field of activity, the Laboratory is second to none. This report has been read with considerable interest not only for its scientific content but also for the extreme diversity of its contents. The new Government Chemist, Dr. D. T. Lewis, is to be congratulated on the presentation of this, his first report.

WILLIAM I. STEPHEN

AMSTERDAM CONFERENCE ON PARTICLE ACCELERATORS

THE January number of *Nuclear Instruments and Methods** is devoted entirely to the proceedings of the Second Accelerator Conference which was held in Amsterdam during October 4–6, 1960. The Conference, which was sponsored by the High Voltage Engineering Corporation, Burlington, Massachusetts, and High Voltage Engineering (Europa) N. V., Amersfoort, The Netherlands, consisted of four sessions, under the chairmanship of Profs. H. Brinkman (University of Groningen), H. H. Staub (University of Zurich), K. Siegbahn (University of Uppsala) and A. Charlesby (Royal Military College of Science), respectively. The seven papers in the first session were devoted to nuclear physics, and dealt with resonance reactions, the generation of positrons in a thick target, desirable linear electron accelerator characteristics for nuclear research, Coulomb excitation, experiments using ultra-fast pulse techniques, nuclear widths and life-times, and colliding beam techniques. Three of the six papers in the session on particle research discussed tandem accelerators. P. H. Rose of the High Voltage Engineering Corporation described two forms of the three-stage tandem accelerator, one with negative ion injection and the other with neutral beam injection. H. E. Gove of the Atomic Energy of Canada, Ltd., gave an account of the tandem accelerator installation at Chalk River and of two particular fields of investigation during 1960. The Chalk River accelerator began operating in February 1959 and has operated continuously on a two-shift a day, five-day week basis since then. The first investigation was concerned with the interaction between beams of heavy ions such as carbon-12, nitrogen-14 and oxygen-16 with targets of nitrogen, oxygen, fluorine, neon, magnesium, aluminium and silicon, and the second with measurements of spins of levels in even-even nuclei lying between oxygen-16 and calcium-40. Recent research work carried out at the Atomic Weapons Research Establishment, Aldermaston, on nuclear reactions using a tandem accelerator and 6-MeV. electrostatic generators was described by K. W. Allen. Oxygen ions accelerated in the accelerator to energies of 10–40 MeV. were used for Coulomb excitation of energy-levels in both rotational and vibrational nuclei and for the measurement of nuclear life-times by Doppler-shift methods. The final paper in the nuclear physics session was given by J. C. Nygard and R. F. Post on recent advances in high-power micro-wave electron accelerators for physics research.

The third and final sessions were devoted to physics and radiation research. The subjects covered included the use of Van de Graaff accelerators, pulsed neutron sources and ion sources. In the industrial applications section, the use of the electron accelerator in solid-state physics, and of high-energy radiation in biochemical and microbiological research were discussed by P. Baruch, and S. T. H. Pinner and W. T. H. Davison, respectively. The report of the proceedings concludes with a most impressive and long list of accelerators (Van de Graaff, micro-wave linear electron and Cockcroft–Walton) which have been installed in various centres all over the world by the High Voltage Engineering Corporation, and associated companies.

* *Nuclear Instruments and Methods*, 11, No. 1: Proceedings of the Second Accelerator Conference, Amsterdam, October 4–6, 1960. Edited by K. Siegbahn. Pp. xii + 256. (Amsterdam: North-Holland Publishing Company). 90*s*.

CONTINENTAL AND OCEANIC DIFFERENTIATION

DR. ROBERT DIETZ'S recent article[1] is a marked contribution to what might be called the oceanographic revolution in geotectonics. One gets the impression that with it a stage has been reached like the last but one in fitting together a jigsaw puzzle, with the various parts of the puzzle having been laboriously assembled, piece by piece, at last seeming to look as if they are all part of one single picture.

What Dr. Dietz has done, effectively, is to combine the notions derived from the recent discovery of oceanographers of mid-oceanic rift ridges and transcurrent faults, with the older pictures, derived from earthquake and gravity measurements, of the pushing up of continental edges along conical thrust faults exposing themselves as mountain ranges, island-arcs and trench systems, according to the ideas of Meinesz and van Bemmelen, and set out specially by Tuzo Wilson. The leading new idea, linked with the still hypothetical mantle convexion systems, is one of expanding oceanic floors, split vertically by latitudinal and circumoceanic transcurrent faults, passing under the continents. This removes many difficulties and makes it possible to envisage a mantle which can expand under some parts of the crust and contract under others at the same time.

XUM

Two major difficulties, however, are still left, and it is to these that I wish to direct attention. In the first place, the general acceptance of an asthenosphere ranging from depths of 120–200 km. below the surface of continents and 70–200 km. below that of oceans does not fit with the presence of deep-focus earthquakes ranging down to 800 km. in depth, with a continuity of thrust planes, marked by their epicentres, with those of the shallow-focus earthquakes. This would seem to imply that it is not only the lower crust, the lithosphere, in Dietz's sense, that moves with the continents but also a rigid upper mantle down to about 900 km., below which the heavier material of reduced density 4 and some degree of plasticity seems to predominate.

To be consistent, the expansion of the oceans must occur down to this depth and from it, too, must proceed the material which by successive crack filling provides for the extension of the oceanic floor and for submarine vulcanicity and particularly for extensive basaltic flows and intrusions. This basaltic material may not derive directly from the lower mantle but may be, so to speak, pumped through the cracked crust from the asthenosphere by the buckling of the upper mantle due to the pressure of the convection currents beneath it. This process, as Gaskell has pointed out, is likely to occur catastrophically rather than continuously and its effects disappear in time by subsidence.

The difficulty of reconciling these two sets of facts turns on the rheological properties of the asthenosphere. It is clearly not a liquid or shear waves would not be transmitted at all but, also, evidently it admits easy creep for earthquakes never originate there, in contrast to the rigidity of the layers above and below it. One possibility is that it is crystallographically heterogeneous in which some minor constituent may be melted thus giving it physical properties similar seismically to poorly consolidated sediments. Such a paste sandwich held between rigid rock above and below might well admit both 45° thrust planes and vertical transcurrent faulting as one rigid body. It may also be that the reason why its upper limit lies higher under the oceans than under the continents is because it is actually hotter there owing to upward convection. The apparent equality of flow of heat through continents and ocean beds might well be an almost accidental compensation, the continental masses making up by their greater radioactive content for the heat flow due, under the oceans, to the closer approach of heated material. These speculations might be amenable to test by heat-flow studies on continental margins and oceanic rifts.

The second difficulty is older and more fundamental. It touches the mechanism of the geochemical differentiation between the sima of the ocean beds and the sial of the continents. Apart from an entirely hypothetical solution like the extraction of the Moon from the Pacific basin, it would seem that this is the result of a progressive tangential concentration of sialic material. Continents seem to grow on their active oceanic margins by the pushing in of an undifferentiated crustal material. But how does the mechanism which Dietz invokes, of the sloping under of the ocean floor, transfer its sial content to the base of the crust? Here, I think, more geochemical analysis is necessary. Something might be found by comparative analytical studies of the clearest example of this under-thrust which occurs in Central America where the Pacific type blocks appear to have been pushed right under the Cordillera of the isthmus and come up again in the Antillean arc, limited by the great transcurrent faults of Cuba and Trinidad.

On both these questions of the real depth of the continental blocks and of the concentration of aluminium in these and not in the oceanic block, experimental methods, both chemical and mechanical, as well as observation and analysis should have a part to play.

J. D. Bernal

Birkbeck College,
University of London.

[1] *Nature*, **190**, 854 (1961).

Regarding Prof. J. D. Bernal's communication, I am pleased to learn that he finds the concept of ocean floor spreading, marking the top of mantle convection cells, at least generally palatable.

Regarding the question of deep-focus earthquakes, I am inclined to favour the idea of the lithosphere extending to about 70 km. both under the continents and ocean basins. This is, of course, derived from isostatic evidence. Below this level, in the asthenosphere, permanent stresses seem not to accumulate. Deep-focus earthquakes down to 800 km. apparently indicate that stresses do accumulate for short periods of at least a few years, but this does not prove their accumulation for thousands or millions of years. Hence deep-focus earthquakes do not disprove the existence of an asthenosphere at moderate depths. As Bernal emphasizes, much depends on the rheological properties of the mantle substance, about which little is known. To describe it as a viscous solid, as a quasi-liquid, etc., must all fail as adequate descriptions.

I agree with Bernal that the apparent equality of flow of heat through the continents and ocean basins may well be a matter of accidental compensation. The surprisingly high flow of heat from the ocean floor with poorly radioactive rocks may be explainable by convection, whereas the heat flow from the highly radioactive granite rocks of the continents must depend on diffusion.

Regarding the sialization of the continents, I visualize that the sima, rising from the deep mantle, contains some juvenile sialic material. By spreading, this 'sial-sima' is eventually slid under the continents. On subsidence of the sima beneath the continent, the sialic fraction gets largely squeezed out (gravitationally differentiated) and is plastered to the underside of a continent. Hence the suggested origin of sial is much like the generally accepted origin of juvenile water. But the mantle substance would tend to be dewatered at the convection cell divergence while it would tend to be de-sialized at the convergences which are necessarily under the continents because of continental drift to the convergence zones. At present, the process seems especially operative around the Pacific, where the mantle seems to be slipping under the continental block and not coupled with it, in contrast to the Atlantic. This added new buoyancy would help account for the recent epeirogenic uplift of the Pacific margin. Thus, I support the concept of new sial being added mostly under the continental block rather than merged laterally and annularly as Tuzo Wilson believes.

Robert S. Dietz

U.S. Navy Electronics Laboratory,
San Diego, 52,
California.

With most of Dr. Dietz's comments on my note I am in agreement. In particular, I find his answer to my second objection, namely, the formation of sial by scraping and squeezing out of undifferentiated 'sial-sima' through the sliding under of the oceanic basement most illuminating.

However, I must still take issue with him on the nature of the upper mantle as witnessed by the existence of deep-focus earthquakes. These cannot, in my opinion, represent merely temporary accumulations of stress in a long-term easy creep material. The location of the conical 45° failure zones must be at least as old as the system of deep sea trenches, island arcs and continental mountain systems they underlie. True, they appear to shift discontinuously by distances of the order of 200 km. as each phase of the orogenic cycle comes into play. However, as Van Bemmelen has demonstrated, the sequence of four parallel thrust planes in the Indonesian arc system goes back at least as far as the base of the Secondary, a period of two hundred millions of years. The most natural explanation of this phenomenon may be similar to that of the development of Luders lines in plastically deformed metals, with old zones of failure hardening up and passing the critical stress on periodically to undistorted material. It seems to me, in any event, somewhat perverse to attribute events occurring 800 km. down in the mantle to causes located in the first 50 km. from the surface; the reverse seems much more likely. If this is so the downward moving convection current under the continental margins might be pictured not as a flow of liquid against a gravitational field through a rigid and highly compressed rock layer, but rather as a displacement downwards of successive blocks of the upper mantle into the presumably secularly softer material of the lower mantle (Fig. 1). Asthenosphere material could thus be imagined as separating into an upper hardened sialic layer under the continental edge and a lower

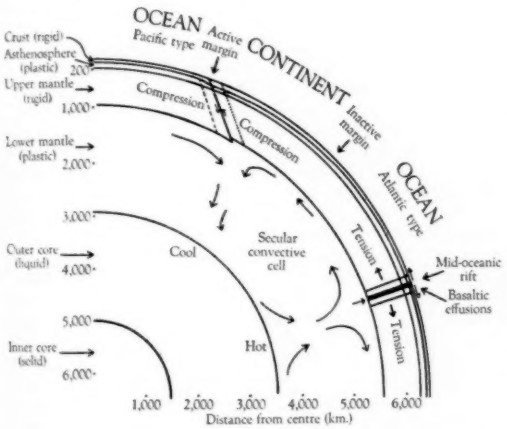


Fig. 1. Schematic Earth section showing one secular convection cell in plastic lower mantle and its reactions on the more rigid upper mantle and through the asthenosphere to the crust. On the right is shown, corresponding to rising mantle current, a mid-oceanic rift through which magma derived from the lower mantle, directly or indirectly, exudes to the surface through successively opened and consolidated tension cracks. On the left is shown, corresponding to a sinking mantle current, an advancing continental edge with trench and mountain building system with the oceanic side of the upper mantle block thrust down into the lower mantle. Between is the inactive continental edge marked only by an increased thickness of the crust and the corresponding thinning of the asthenosphere

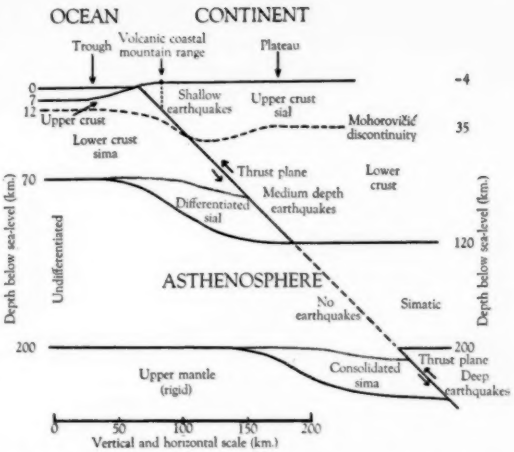


Fig. 2. Section of an idealized continental thrust plane system illustrating Dietz's hypothesis of continental sialic differentiation. The upper block of asthenospheric sial will in the actual case not be so clearly demarcated from the basal crust as is shown, nor will the corresponding block of sima with the sinking upper mantle. The former, as it is differentiated, tends to rise vertically to jam with, and push up, granite batholiths. The vulcanicity associated with the mountain building is assumed to arise from the activity of the thrust plane itself

hardened simatic layer filling in and overlying the depressed former upper mantle surface (Fig. 2).

These remain very tentative suggestions but may serve to pull together in one coherent whole the evidence derived from recent geophysical and oceanographic studies.

J. D. Bernal

In two recent papers R. S. Dietz[1] and H. H. Hess[2] have made similar proposals in which, guided by new discoveries made about palæomagnetism and the ocean floors, they have combined features of several older theories into one which appears to fit many observations. J. D. Bernal has welcomed these views (first communication) and commented on particular points and I should like to do the same.

Essentially, Dietz and Hess agree that major convection currents flow in the mantle, as long advocated by Arthur Holmes, F. A. Vening Meinesz[3] and many others.

These currents are held to rise under the active mid-ocean ridges and sink under the belts of active mountains and island arcs which lie along some continental margins. The locations of these are well known and so it is possible to sketch on a globe the approximate directions of motion of the upper surfaces of these currents flowing from the mid-ocean ridges towards the active continental mountain systems.

Mid-Ocean Ridges

A current rising under the mid-Atlantic ridge provides an explanation for the earlier views of A. Wegener[4] and A. L. Du Toit[5] that the opposite coasts of the ocean have moved apart. The presence of this current can also explain the central position of the ridge.

The westward current bears the Americas towards and over the limb where the current is supposed to descend beneath the Cordillera and the Andes,

while a current flowing eastwards can be assumed to converge towards the Alpine-Himalayan mountain belt from the north-west and the south-west. The extension of this concept to the ridge through the Southern Ocean poses a difficulty. From the Atlantic the ridge passes south of Africa and Australia to reach the Americas, but a current spreading south from this part of the ridge would flow towards Antarctica from nearly every direction and have no sink into which to descend. The only compressional mountains in Antarctica lie along the antarctic peninsula (Grahamland) and these are scarcely adequate. To escape the dilemma one must suppose that, instead of a current flowing south from the ridge, the ridge itself is migrating northwards and that the ring which it forms around most of Antarctica is expanding in radius. If so, the current flowing northwards on the other side of the ridge is moving at twice the normal rate (taken as of the order of 1 cm./yr. by both Dietz and Hess).

This would cause a relatively rapid northward movement of all the southern continents except South America, a fact which is borne out by palæomagnetic observations. Blackett, Clegg and Stubbs[6] have recently shown that for the past 2×10^8 years Africa, India, and Australia have all been moving north at rates of 2–8 cm./yr.

It will be observed that, since the mid-ocean ridge meets the west coast of South America and does not pass south of it, there is not the same reason for South America to have moved north. Nevertheless, its fit with Africa suggests such motion. It would be useful to have palæomagnetic data for South America.

(*Note added in proof.* E. Irving has directed my attention to palæomagnetic work by K. M. Creer[16,17] on the Jurassic (Serra Geral) lavas of Uruguay and southern Brazil which suggests that the Brazilian Shield has not changed appreciably in latitude since the Jurassic—a result consistent with the view outlined here.)

A similar outward motion may also be taking place in the mid-ocean ridges which in the South Atlantic, Southern and Indian Oceans nearly surround Africa. If one is to believe that the African rift valleys are due to spreading, this expansion must be happening.

A corollary of this expansion of the ring-shaped ridges around Antarctica and Africa is that, since they cannot expand indefinitely, changes must occur occasionally in the location of the currents in the mantle.

The ridge across the Pacific, called the East Pacific rise, extends to the Gulf of California[7]. Seismicity and topography suggest that two branches reach Juan Fernandez and Galapagos Islands, but these have not been fully investigated. If these branches are also upwellings, each ridge will induce a faster flow northwards, for there is no sign of any compressional features between them. Following the previous argument there should be no movement of Cape Horn away from Antarctica ; but north of Juan Fernandez there should be a motion twice that of the convection-rate, north of Galapagos four times that rate, and off California six times that rate. This may provide an explanation of the lack of any shearing or isthmus across the Scotia Sea, the anti-clockwise rotation of the Pacific basin and the strong motion along the San Andreas fault.

The current flowing east from the East Pacific rise meets that from the Atlantic under the Andes to produce their present uplift and activity, but Menard's proposal that in the United States and Canada the East Pacific rise now lies along the most active part of the Cordillera, implies that a former downward current has been replaced by an upward one which must have involved some drastic tectonic adjustments. One possible consequence of removing the downward current would be to cause North America to move westward relative to South America. This could have been a factor in producing the large shear faults in the Caribbean and those recently discovered in the floor of the Pacific off North America of which Menard[7] gives details. Unfortunately their age is unknown.

The current flowing north-west from the East Pacific rise presumably meets the current flowing south-east from the ridges in the North Atlantic Ocean and Arctic Sea. The resulting downflow and compression can be held to have produced the east Asian arcs in much the manner postulated by Scheidegger[8]; indeed, no other explanation has yet been offered why failure should take the form of circular arcs with their centres tending to lie on continental margins. Thus some features of the contraction theory are preserved. This movement of the Pacific basin north-west past the end of the Indonesian-Melanesian belt of mountains and island arcs fits the shearing observed between the Philippines and New Zealand[9].

Hess suggests that the East Pacific rise may have only been formed in mid-Cretaceous time when the location of a rising convection current changed to its present position from an earlier one lying at right angles in a west-north-westerly direction from the Society to the Caroline Islands. Before this change, the orogenetic forces would have been quite different from to-day and the Cordillera would have been under strong compression and uplift. The change may well have coincided also with the formation of several new island arcs in the Pacific. The Kuril, Aleutian and Mariana, Yap and Palau Islands appear to be of about that age.

Continental Mountain Systems

The concept that the circum-Pacific and Alpine-Himalayan mountain systems are due to compression over downward flowing limbs of huge convection cells is straightforward except for the complication already mentioned in California and Dietz's enigmatic statement that a rift is opening in the Mediterranean. It appears rather to be closing. This theory offers an explanation of two formerly puzzling features of older arcs. The first is that it was never apparent why island arcs, so symmetrical in youth, as for example the Kurils, should lose this with age, for example in the Andes. It now appears likely that the Andes formed as arcs off the coast of South America and that these arcs have been overrun and swept up by the westward motion of the continent. The contrast between the position of deep-focus earthquakes under the heart of the South American continent[10] and off the margin of east Asia is another indication of this. To be thus caught up and overridden appears to the fate of arcs.

Another feature, which was hard to explain by earlier theories and which appeared to be contrary to uniformitarianism, is that, whereas the active mountains form one continuous T-shaped pattern, older mountain systems only form short lengths. It is difficult to fit the Hercynian Appalachian mountains

together into a single system and quite impossible to do it for Precambrian orogenies.

Relations to Older Orogenetic Theories

Besides admitting of some features of compression in mountains, the new theory admits of continental growth, both by sweeping up sediments on the ocean floor and by the emission of andesitic lavas. This growth along some continental margins implies that coasts separated by rifting need not necessarily fit if later brought together again.

Not only does this marginal growth make it difficult to find the coasts which formerly fitted together, but also old theories of continental drift included the notion that all continents had formerly been united into one or two. This idea, which would have involved untenable distortion of continents, seems unnecessary. The blocks of continents should rather be thought of as sometimes moving together in one place and sometimes splitting and moving apart at another place, always producing scattered patterns.

In one respect the break-up of continents does not seem to be quite random. There appears to be a tendency for them to split along former mountain systems. Thus on the Atlantic coasts of both Africa and South America there seem to be parts of mountain systems formed at the end of the Precambrian or in early Palæozoic time, suggesting that a single system split longitudinally. As pointed out by M. S. Krishnan[11], there is a similar correspondence between the late-Precambrian rocks of East Africa, Madagascar, Ceylon and southern India. The East African rift valleys follow rather closely the boundary between the Mozambique belt and older Precambrian provinces in the interior of Africa. If Menard is correct, the East Pacific rise is starting to split the Cordillera in California.

If convection currents are continually stirring the mantle, they would ensure a uniformity in its composition which would fit the views of Russell and Farquhar on the source of lead ores[12].

A point about possible convection currents which has been generally overlooked concerns the change in viscosity of the mantle with depth. Due to the thermal gradient this is likely to be large. If so, the cross-section of convection cells should be much greater near the surface than near the core of the Earth. Diagrams and discussions of convection in the Earth rarely consider this.

Movements of continents would cause the principal moments of inertia of the Earth to change and would induce polar wandering. This process would be particularly effective where two continents pile up against one another to great elevations as north of India. If such compression proceeds at the rate of a few cm./yr., it would produce hundreds of km. of shortening in ten million years. Since no mountains are compressed more than this, some factor must either change the pattern of convection cells within ten million years after continents come into contact or else a new process must arise by which convection currents begin to shear beneath continents. Three possible cases are worth considering. If some continents during certain periods have had a current flowing beneath them, whereas at other times they have been passively borne along with the current, it may be possible to find some geological phenomena, such, for example, as periods of dike formation, associated with one motion and not with the other. Another alternative is that the convection currents may be stopped or even reversed by the piling up of heat-producing continental material. To test this more heat-flow measurements are needed over mountains. It is tempting to relate the somewhat symmetrical arrangement of currents about the Earth's axis to dynamical control as is the case in the Earth's core. This symmetry was the basis of the tetrahedral hypothesis and has been translated into modern terms of ridges and mountains[13]. If this relation were true then polar wandering would affect the location of convection currents, but the evidence about the rate of polar motion suggests that it is too slow to effect changes in mountain building currents within periods of the order of ten million years. It is also doubtful if the forces are strong enough to make this linkage important. A reason for periodically changing the location of convection cells by large amounts is still needed.

The collision of continents might be expected to produce migrating waves of uplift which could catch klippe of sedimentary rocks on their advancing crests and carry them forward like surfboards on waves, stranding them when the uplift subsided. This offers a possible expanation, heretofore lacking, of some isolated and far-reaching overthrusts.

Persistence of Continental Freeboard

Dietz and Hess both recognize that continents and ocean basins are in isostatic equilibrium and that no large part of a continent can very long remain high because erosion and the rise of its roots will bring it down nearly to sea-level. They also recognize that the constant additions of juvenile water and lava from within the Earth make the problem of raising the level of the continents and of thickening them equally important, but more difficult. They suggest three mechanisms, of which Dietz states that continents "tend to buckle, which accounts for alpine folding, thrust faulting and similar compressional effects", that clastic detritus swept into the deep sea "is carried slowly towards, and then beneath the continents, where it is granitized and added anew to the sialic blocks", and that "new buoyancy is added to continents through the gravitative differentiation from the mantle".

The first two are true under mountains, but not under shields, where the crux of the problem lies. If for the sake of argument we adopt Hess's view that 4×10^9 years ago the continental blocks had half and the oceans a third of their present volumes, and that both have grown at a uniform rate, it can be shown that in Cambrian time, 6×10^8 years ago, the oceans would have been on the average 0·7 km. shallower and the continents 3·3 km. less thick than to-day. Dietz and Hess both maintain that to avoid being drowned beneath the sea, not only mountains but also the crust beneath plains must have been thickened by some such amounts.

The almost flat-lying Palæozoic rocks which cover so much of the interior plains of Canada and United States provide evidence that buckling of the shield has not been the cause of any post-Cambrian thickening of the plains.

To suppose that sediments were carried down along continental margins, metamorphosed and that they rose again is a very satisfactory explanation of the gneissic batholiths in marginal mountains, but what evidence is there that sediments are carried far under continents and that this process operates to uplift

plains? In the western Andes there are many batholiths to which the above explanation might apply, but there is nothing to suggest that batholiths are rising above the foci of the deep earthquakes in the Upper Amazon or Parana-Paraguay valleys. These valleys are at low elevations and are not being uplifted. Hess's argument about the spreading of mountain roots fails for the same reason.

The third concept, that of differentiation of acid rocks in the mantle and their rise, is one which I have supported as an explanation of andesitic volcanism in the continental mountain systems; but what evidence is there that any such process has added about 3 km. to the thickness of the interior plains of North America since the Cambrian? The only evidences of igneous activity there are a few nepheline basalt volcanoes of Cretaceous age, forming the Monteregian hills near Montreal, small carbonatite intrusions like the ten between Montreal and Lake Superior[14], very small pipes of ultra-basic rocks as in Kansas, some crypto-volcanic structures and a few other igneous intrusions, all small, isolated and rarely siliceous. Most of these rocks are not acid differentiates and most of them can only have come from the mantle. If these intrusions could find their way to the surface, and if 3 km. of acid rocks had been added to the base of the crust during the same period, surely there would be more signs of the acid rocks. All this evidence suggests that continents grow at their margins. Although it is often stated that continental shields thicken, there is a lack of evidence that this has happened, at least since early Precambrian time.

The impasse is avoided if no new material is being added from the interior of the Earth, but this is contrary to several sound arguments. Another possibility is that the Earth is slowly expanding. A rate of a few mm./yr. would suffice to maintain continents above sea-level. This would not interfere with and indeed might assist the other orogenetic processes described. As Dicke[15] has pointed out, this is a matter of fundamental importance in physics and cosmology and one which it is very hard to prove or disprove.

J. Tuzo Wilson

Institute of Earth Sciences,
University of Toronto,
Toronto, Canada.

[1] Dietz, R. S., *Nature*, **190**, 854 (1961).
[2] Hess, H. H., preprint from Goldberg, E., Munk, W., and Iselin, C. O'D., *The Sea, Ideas and Observations* (Interscience Publishers, New York, in the press).
[3] Vening Meinesz, F. A., *Geol. en Mijnbouw*, New ser., **14**, 373 (1952).
[4] Wegener, A., *The Origin of Continents and Oceans*, Trans. by Serl, J. G. A. (Methuen and Co., Ltd., London, 1924).
Du Toit, A. L., *Our Wandering Continents* (Oliver and Boyd, Edinburgh, 1937).
[5] Blackett, P. M. S., Clegg, J. A., and Stubbs, P. H. S., *Proc. Roy. Soc.*, A, **256**, 291 (1960).
[7] Menard, H. W., *Science*, **132**, 1737 (1960).
[8] Scheidegger, A. E., and Wilson, J. T., *Proc. Geol. Assoc. Canada*, **3**, 167 (1950).
[9] Jacobs, J. A., Russell, R. D., and Wilson, J. Tuzo, *Physics and Geology*, 309 (McGraw-Hill Book Co., Inc., New York, 1959).
[10] Gutenberg, B., and Richter, C. F., *Seismicity of the Earth* (Princeton Univ. Press, Princeton, 1950).
[11] Krishnan, M. S., *Proc. Ind. Acad. Sci.*, **52**, 49 (1961).
[12] Russell, R. D., and Farquhar, R. M., *Lead Isotopes in Geology* (Interscience Publishers, New York, 1960).
[13] Wilson, J. Tuzo, *Nature*, **185**, 880 (1960).
[14] Parsons, G. E., *Ontario Dept. Mines, Geol. Rep. No.* 3 (1961).
[15] Dicke, R. H., *Amer. Sci.*, **47**, 25 (1959).
[16] Creer, K. M., *Ann. Geophys.*, **14**, 373 (1958).
[17] Creer, K. M., Irving, E., Nairn, A. E. M., and Runcorn, S. K., *Ann. Geophys.*, **14**, 492 (1958).

CARBONYL CONTENT OF THE VITRAIN COMPONENT OF BITUMINOUS COALS*

By Dr. K. LITTLEWOOD and M. T. MONAGHAN

Department of Fuel Technology and Chemical Engineering, University of Sheffield

It is generally recognized that oxygen occupies a position in the structure of coal comparable with that of carbon. Consequently, much work has been carried out in order to determine the percentage of oxygen contained in the various functional groups in coals of a wide range of carbon content. A summary of the methods used and the results obtained has been given by Blom *et al.*[1].

Of the methods used previously for determining the carbonyl fraction, that which has found widest application involves the use of excess phenylhydrazine[1,2]. In this method the finely divided coal is treated with phenylhydrazine at 100° C. in an aqueous medium, followed by volumetric determination of the excess reagent. Although fairly consistent results have been obtained the method suffers from several disadvantages which make the results subject to error. For example, both coals and humic acids react with compounds containing the NH_2 group (for example, aqueous ammonia at 100° C.[3], ethylenediamine[4], etc.), forming compounds which are not hydrolysable.

Other errors result from the fact that many quinones are reduced by phenylhydrazine so that they do not yield any hydrazone. In addition, the presence of aromatic rings inhibits the complete formation of the hydrazone[5].

In view of these difficulties, a method based on the reaction of carbonyl compounds with hydroxylamine was evolved[1]. Coal of known nitrogen content is treated with hydroxylamine in pyridine at 90° C. for 20 hr. The nitrogen content of the coal remaining is then determined before and after hydrolysis with sulphuric acid. The hydrolysed oxime is determined as ammonia. In every instance it was noted that more hydroxylamine was taken up than could be split off by hydrolysis. The anomalous results were attributed to reactions of hydroxylamine with non-carbonyl substances and to the fact that the oximes of some carbonyl compounds are not readily hydrolysable.

As neither of the above techniques was completely reliable, it was decided to investigate the possible application of the specific reagent, trimethylacetoaminohydrazide hydrochloride (Girard's reagent-*T*)[6], to the determination of the carbonyl content of vitrains. Girard's reagent is a white, crystalline

* Based on a paper presented at the fourth International Conference on Coal Science, Le Touquet (May 30–June 2). See p. 116 of this issue.

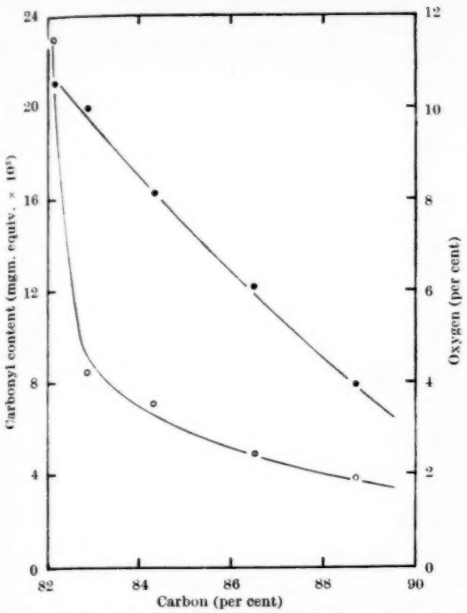


Fig. 1. Relationship between carbonyl content, oxygen content and coal rank. ○, carbonyl; ●, oxygen

solid which is very soluble in water and organic solvents containing a hydroxyl group. The reagent has a great affinity for ketonic carbonyl compounds and reacts with them quickly and quantitatively even when they are present in very small amounts[7–10].

The function of the reagent is to produce a water-soluble derivative by a condensation reaction with the carbonyl compound which can then be separated from the remaining inactive material. The carbonyl fraction thus removed can be regenerated by acid hydrolysis and afterwards estimated by conventional methods. The reactions involved are illustrated in the following reactions:

$$R_1R_2C{=}O + (CH_3)_3\overset{Cl^-}{\overset{|}{\overset{+}{N}}}CH_2CONHNH_2 \longrightarrow R_1R_2C{=}NNHCOCH_2\overset{Cl^-}{\overset{|}{\overset{+}{N}}}(CH_3)_3$$

$$R_1R_2C{=}NNHCOCH_2\overset{Cl^-}{\overset{|}{\overset{+}{N}}}(CH_3)_3 \xrightarrow[\text{hydrolysis}]{\text{acid}} R_1R_2C{=}O$$

Thus the obvious advantages of Girard's reagent are: (1) it reacts exclusively with carbonyl compounds; (2) it extracts the carbonyl compounds as water-soluble derivatives which are easy to manipulate.

The analytical data of the vitrains used are summarized in Table 1 and the experimental procedure

Table 1. Analytical Data of the Vitrains

No. of coal	N.C.B. code No.	Water per cent	Ash per cent	Analysis (Parr basis) S (d.a.f.) per cent	C per cent	H per cent	N per cent	O (diff.) per cent	V.M. (d.a.f.) per cent
1	301	6·40	0·97	0·68	88·67	5·38	2·04	3·91	23·53
2	501	1·66	0·06	1·14	86·49	5·44	1·98	6·09	34·50
3	602	6·83	1·22	1·26	84·34	5·05	1·92	8·11	37·59
4	701	5·09	5·43	0·97	82·88	5·36	1·78	9·98	33·41
5	802	6·99	1·78	1·18	82·16	5·37	1·90	10·57	40·43

is as follows: About 2·0 gm. dry vitrain (− 200 mesh B.S. sieve) were weighed accurately and then transferred to a 500-ml. flask to which were added 100 ml. absolute ethyl alcohol, 10 ml. glacial acetic acid and 5 gm. Girard's reagent-*T*. After boiling under reflux for 24 hr., the solution was cooled by the addition of 300 ml. distilled water containing sufficient caustic soda to neutralize 90 per cent of the acetic acid present. After allowing the insoluble material to settle, the solution was filtered through a weighed sintered glass crucible (porosity *G*4); the residue was washed and dried at 110° C. until of constant weight.

In order to hydrolyse the water-soluble derivative, 10 ml. concentrated sulphuric acid were added to the combined filtrate and washings and the resulting solution was warmed on a water-bath at 70° C. to complete the hydrolysis. The solution, after cooling, was added from a burette into an excess of 2,4-dinitrophenylhydrazine. The mixture was allowed to stand for 1 hr. to ensure complete precipitation of the hydrazone. The resulting hydrazone was filtered through a weighed sintered glass crucible (porosity *G*4), washed and dried at 110° C. until of constant weight.

The results obtained by determining the carbonyl content by reaction with Girard's reagent-*T* followed by hydrolysis and estimation with 2,4-dinitrophenylhydrazine are given in Table 2. The results are quoted in terms of mgm. 2,4-dinitrophenylhydrazone (column 3) and have been converted to mgm. equivalents of C=O in column 4.

Table 2. Carbonyl Contents of the Vitrains

Coal	Carbon (per cent)	mgm. dinitrophenyl-hydrazone/gm.	mgm. equiv.* C=O/gm.
301	88·67	6·0	0·03
501	86·49	10·0	0·05
602	84·34	15·0	0·07
701	82·88	17·5	0·08
802	82·16	48·0	0·23

* Maximum values.

The vitrains which were investigated ranged from 82 per cent carbon (10·6 per cent oxygen) to 89 per cent carbon (4 per cent oxygen). The expected decrease in carbonyl content with increasing coal rank, that is, decreasing oxygen content, is clearly demonstrated in Fig. 1. The sensitivity of the reagent in detecting the differences in carbonyl contents of coals lying in such a narrow range of carbon content is of particular interest and is indicative of the specificity of the reagent. Other investigators suggest that this trend is correct although this has not been demonstrated previously.

The use of Girard's reagent in conjunction with 2,4-dinitrophenylhydrazine has been extended to give an indication of the weight average molecular weights of the ketones involved. Full details of this are to be published elsewhere.

We wish to thank Prof. M. W. Thring for his permission to submit this article.

[1] Blom, L., Edelhausen, L., and van Krevelen, D. W., *Fuel*, **36**, 135 (1957).
[2] Strache, H., and Brandl, A., *Brennstoff-Chem.*, **7**, 341 (1926).
[3] Fuchs, W., and Leopold, H., *Brennstoff-Chem.*, **8**, 73 (1928).
[4] Bangham, D. H., and Dryden, I. G. C., *Fuel*, **29**, 291 (1950).
[5] Wild, F., *Estimation of Organic Compounds*, 140 (Camb. Univ. Press, 1952).
[6] Girard, A., and Sandulesco, G., *Helv.*, **19**, 1095 (1936).
[7] Rodd, E. H., *Chemistry of Carbon Compounds*, **11** B, 930 (Elsevier Publ. Co., 1953).
[8] Talbot, N. B., Butler, A. M., and MacLachlan, E., *J. Biol. Chem.*, **132**, 595 (1940).
[9] Callow, R. K., *et al.*, *Biochem. J.*, **29**, 1424 (1935).
[10] Wolfe, J. K., Hershberg, E. B., and Fieser, L. F., *J. Biol. Chem.*, **136**, 653 (1940).

FORMATION OF CRACKS IN ICE PLATES BY THERMAL SHOCK

By L. W. GOLD

Snow and Ice Section, Division of Building Research, National Research Council, Canada

IN work on crack formation in multi-grained ice during creep under constant compressive load[1], the question arose as to whether the direction of the formation of a crack depended on the crystallographic orientation of the grains in which it formed. Experiments were undertaken to investigate this possible dependence. In one set of experiments a thermal shock was applied to the flat surface of an ice plate in such a way that the resulting thermal stress exceeded the cohesive strength of the ice almost instantaneously.

The first observations were made on irregularly shaped ice plates prepared from single crystals grown by a technique described by Landauer[2]. The plates were about 10 cm. in length and width and about 2 cm. thick. They were prepared with a milling machine using a helical cutter about 15 cm. long. Some plates were machined so that the axis of hexagonal symmetry, or 'c' axis, of the ice crystal was normal to the surface and some so that the 'c' axis was parallel to the surface. After machining, the plates were stored in kerosene at − 10° C. for at least 24 hr. Earlier work on the elastic and strength properties of ice had shown that immersion of the ice in kerosene for periods of up to 30 days or more did not appear to affect these properties.

Thermal shock was applied by bringing the smooth surface of an ice plate, at − 10° C., in contact with the smooth flat surface of a brass plate cooled to below − 45° C. with dry ice. To ensure good thermal contact, the brass plate was immersed in kerosene just deeply enough to ensure that a very thin film of the liquid lay over the surface to which the ice plate was applied.

When the ice plates were brought into contact with the brass plate, cracks formed very quickly, certainly within 1 sec. For this stress condition, outside the region influenced by the edge the principal stresses are equal and the shear stresses are zero on any plane parallel to the ice surface. Therefore very little, if any, creep could have occurred in such planes or perpendicular to them in the short time that it took for the cracks to form.

The crack pattern which developed was found to depend almost entirely on the crystallographic orientation of the ice with respect to the shocked

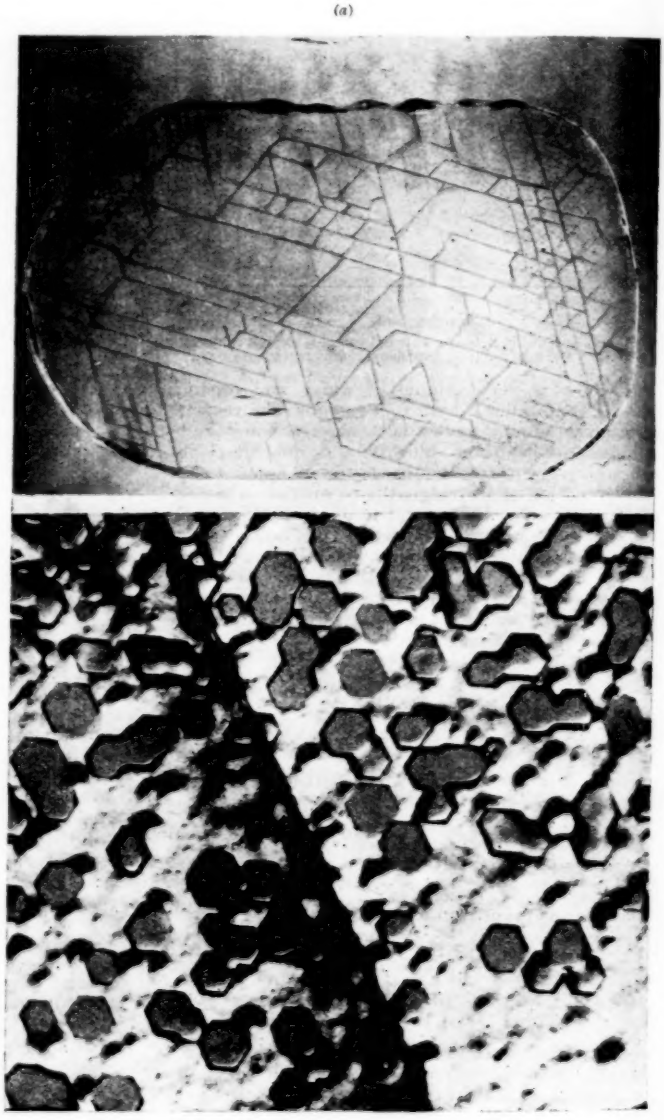
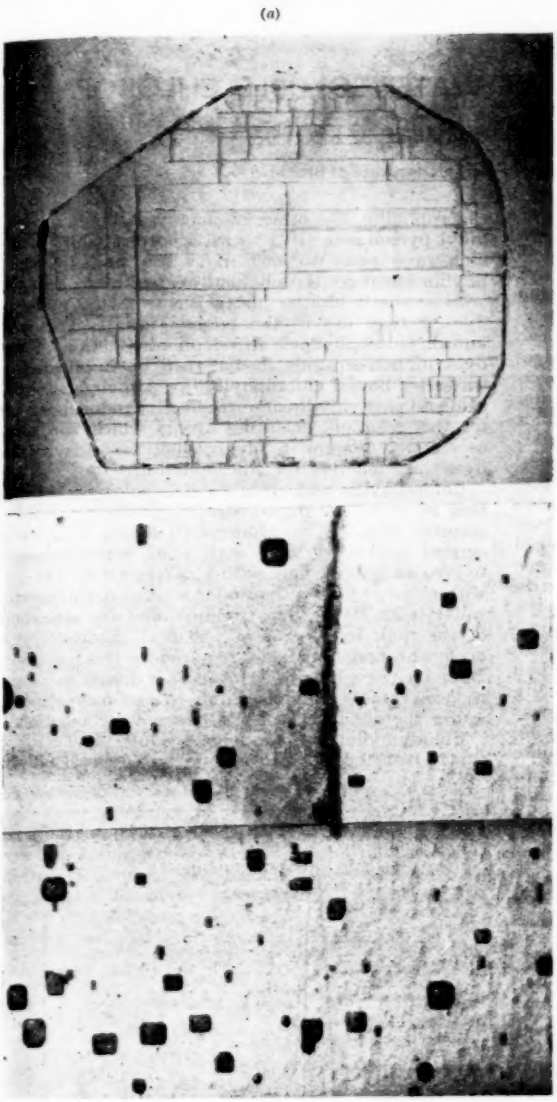


Fig. 1. (*a*) Crack pattern for axis of hexagonal symmetry perpendicular to surface of plate; (*b*) orientation of cracks with respect to hexagonal etch pits. Crack is line running from upper left to lower right. (× 50)



(*a*)

(*b*)

Fig. 2. (*a*) Crack pattern for axis of hexagonal symmetry parallel to surface of plate; (*b*) orientation of cracks with respect to rectangular etch pits. Basal plane of ice perpendicular to surface and parallel to the bottom edge of the photograph. (× 40)

surface. An example is shown in Fig. 1*a* for the case where the axis of hexagonal symmetry is perpendicular to the plate surface. The crack pattern is a system of equilateral triangles. As shown in Fig. 1*b*, the trace of the cracks on the surface was found to be parallel to the sides of hexagonal etch pits obtained by a technique described by Higuchi[3]. This shows, accepting Higuchi's conclusions on the orientation of the pits with respect to the crystallographic orientation, that with the symmetry axis perpendicular to the surface, there is a preference for the trace of the cracks at the surface to be parallel to the crystallographic '*a*' directions.

The rectangular crack pattern which develops when the symmetry axis lies in the plane of the plate surface is shown in Fig. 2*a*. Fig. 2*b* shows that the edges of the cracked segments are parallel to the sides of the rectangular etch pits that form on an ice surface with this orientation. The long dimension of the cracked segment was usually perpendicular to the crystallographic '*c*' direction, that is, this edge was normally in the basal plane.

The edges of each plate were cut in an irregular shape to see if this would have any influence on the crack pattern. It was observed, as can be seen in Figs. 1*a* and 2*a*, that only very close to the edge did the boundary conditions have an influence.

Figs. 1*b* and 2*b* show that there is a preference for the trace of the crack at the plate surface to be in the planes containing the '*a*' and '*c*' crystallographic directions. Etch pits formed on surfaces machined perpendicular to the plane of the crack and to the plate surface showed that near the plate surface the cracks tend to be parallel to the planes containing the '*a*' and '*c*' directions, but often curve out of these planes farther into the interior of the ice. This is considered to be due to the tendency for the cracked segments to curl under the tensile stresses parallel to the plate surface. This curving of the crack may be aided by the fact that the maximum tensile stress at the root of a crack is not perpendicular to the plane of the crack but at an angle to this plane[4]. The shaded area parallel to some of the cracks is due to the curving of the crack below the surface. If the thermal shock is severe and long enough, some of the crack segments will separate from the plate in a way similar to the spalling of concrete.

The experiments have now been extended to multigrained ice of average grain size as small as 2 mm. and with the crystallographic orientation of the grains at various angles to the surface. The preference for the trace of the cracks at the surface to be parallel to the planes containing the '*a*' and '*c*' directions is maintained for all grain sizes observed so far, a crack often changing direction abruptly as it passes from one grain to the next.

Some refinements have been made in the experimental technique. By using ice instead of brass for the cold plate and making both plates quite thick the change in temperature at the surface can be calculated and readily controlled and expressions obtained for the dependence on time of the temperature in the ice plates and the associated thermal stress. Using these expressions, a cohesive strength of 40–50 kgm./cm.^{2} has been obtained for ice for the particular stress condition applied. The experiments and their analysis are continuing and will be reported in full when completed.

[1] Gold, L. W., *Canad. J. Phys.*, **38**, 1137 (1960).
[2] Landauer, J. K., *Res. Rep.*, No. 48, Snow, Ice and Permafrost Research Establishment, Corps of Engineers, U.S. Army (1958).
[3] Higuchi, K., *Acta Metal.*, **6**, 636 (1958).
[4] Williams, M. L., *Trans. Amer. Soc. Mech. Eng., J. App. Mech.*, **79**, 109 (1957).

PHOTOCHEMICAL PROPERTIES OF WATER-SOLUBLE CHLOROPHYLL

By Prof. GERALD OSTER and SUSE B. BROYDE

Department of Chemistry, Polytechnic Institute of Brooklyn

THE water-soluble chlorophyll, potassium chlorophyllin, obtained by saponification of chlorophyll with alkali, has been known for more than fifty years[1], yet the photochemistry of this interesting pigment has not been investigated. Since chlorophyllin is soluble in water, its photochemical properties can be compared directly with other water-soluble dyes the photosensitizing action of which for oxidations and reductions have been studied in detail[2]. Water is a more natural *milieu* than are the organic liquids which are required to dissolve chlorophyll. Water-soluble dyes will bind to high-polymer substrates[3] with dramatic changes in photo-chemical properties[4,5] and chlorophyllin would be expected to behave similarly. Furthermore, since chlorophyll is in the bound state in the living plant, the photochemical properties of bound chlorophyllin may have some immediate relevance to the photochemical steps in photosynthesis. Another reason for studying the photochemistry of chlorophyllin is that it has some structural differences from chlorophyll and hence theories as to the reactive groups can be tested.

Chlorophyll *a* was obtained chromatographically from spinach extract[6] and the dried pigment was dissolved in benzene and diluted 1 : 14 with pentane. This solution was shaken with 7 per cent methanolic potassium hydroxide to saponify the chlorophyll. The methanolic solution of potassium chlorophyllin *a* was placed under vacuum in order to remove the solvent and the material was stored dry. The photochemical investigations were carried out with chlorophyllin in 0·6 *M* phosphate buffer at *p*H 7·0.

Treatment with methanolic potassium hydroxide causes the material to be soluble in water. The phytol and methyl esters are saponified. Furthermore, the cyclopentanone ring (ring V in the chlorophyll structure) is broken so that three carboxylate groups result. The magnesium is retained at neutral and at basic *p*H values.

The infra-red spectra of chlorophyllin (potassium bromide pellets and measured in a Perkin-Elmer model 21 spectrophotometer) do not have the 1611 cm.$^{-1}$ band associated with porphyrins containing a cyclopentanone ring[7] and ascribed to the C=C bond of this ring in its enolic form[8]. The two principal absorption bands in the visible spectrum of chlorophyllin (Fig. 1), measured in a Cary spectrophotometer, are at shorter wave-lengths than chlorophyll *a*, and the satellite on the short wave-length side of the Soret band is absent. Similar changes in the visible spectrum are observed in one of the fractions of allomerized chlorophyll where the cyclopentanone ring is believed to have been transformed[8]. Other losses in detail in the absorption spectrum further indicate that the molecule resulting from cleavage of ring V is less rigid. The strong red fluorescence of chlorophyllin has its maximum at 646 mμ while chlorophyll *a* has its maximum fluorescence at about 20 mμ farther into the red part of the spectrum.

Chlorophyllin in the bound state is a good photosensitizer ; free in solution, it is much less effective. Chlorophyllin, like other anionic dyes[3], binds to polyvinyl pyrrolidone (PVP) with a spectral shift of the long-wave peak to 660 mμ. The bound chlorophyllin sensitizes the photoautoxidation of *para*- and *ortho*-aromatic amines just as do triphenyl methane dyes if they are in the bound state[10]. The well-known chlorophyll-sensitized photoreduction of azo dyes in non-aqueous media[11] can be duplicated in water by bound chlorophyllin[12]. Although ascorbic acid and phenylhydrazine are effective reducing agents for this reaction, chelating agents which can serve as electron donors in photoreductions of synthetic dyes[13] are inactive for chlorophyllin.

Chlorophyllin undergoes the Krasnovsky reaction[14], that is, reversible photoreduction in the presence of ascorbic acid. With chlorophyll *a* this reaction is carried out in pyridine with some water present[15] to give an intermediate with a maximum at 523 mμ ; with chlorophyllin *a* the major solvent constituent is water (with 10 per cent pyridine) and the maximum of the pink solution is at 550 mμ. Evidently the cyclopentanone ring is not required for this reaction[16]. Bound chlorophyllin, on the other hand, is photoreduced rapidly and irreversibly to a colourless species under the conditions of the Krasnovsky reaction.

Chlorophyllin, on standing in aqueous solution, is slowly transformed into a colloidal species, and this

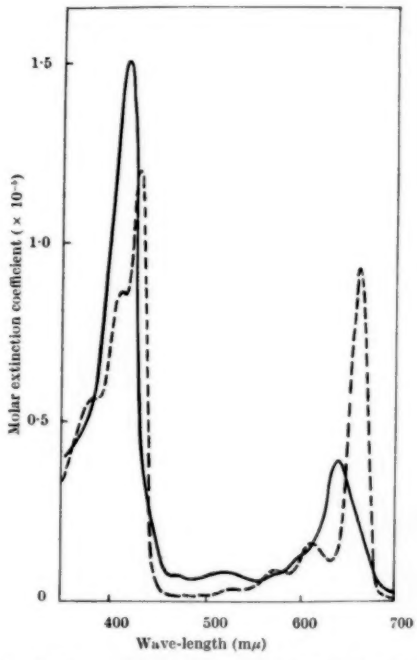


Fig. 1. Spectra of chlorophyllin *a* (continuous line) and chlorophyll *a* (dotted line)

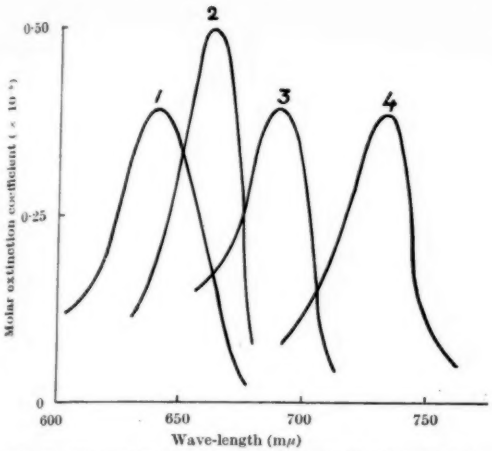


Fig. 2. Changes in long wave-length peak of chlorophyllin: 1, freshly prepared in water; 2, addition of PVP to No. 1; 3, after illumination of No. 2; 4, long standing of No. 1 (reverts to No. 3 on addition of PVP and to No. 1 on addition of alkali)

transformation is accelerated by light or heat. The system is non-fluorescent and absorbs at 735 mμ (Fig. 2) as is also the case for suspensions of chlorophyll microcrystals[17]. This suggests that the state of aggregation, rather than the difference in details of structure, determines the position of this peak. Addition of PVP or of water-miscible organic solvents disperses the colloid to produce a strongly fluorescent solution with an absorption maximum at 688 mμ. This latter species can be obtained directly by binding chlorophyllin to PVP and exposing to light (Fig. 2). Both the colloidal suspension and the solution absorbing at 688 mμ revert to the original chlorophyllin solution when the system is rendered alkaline. The species which forms colloidal suspensions in water has an infra-red band at 1,730 cm.$^{-1}$ as compared with the analogous band of chlorophyllin itself at 1,680 cm.$^{-1}$. These facts suggest that the water-insoluble species results from a dimerization of the carboxylate groups in chlorophyllin. The existence of a dimer of chlorophyll *a* absorbing at about 682 mμ has been deduced from difference spectra of chlorophyll solutions[18].

The photochemical reactions of chlorophyllin described here supplement the extensive work already carried out on chlorophyll[19], but new features are apparent. Our results show that: (*a*) photochemical activity of a chlorophyll derivative does occur in predominantly aqueous media; (*b*) the spectra of aggregates of chlorophyllin are the same as those of chlorophyll although there are differences in molecular structure; (*c*) certain spectral features and photochemical reactions occur for bound pigment but not for the molecules free in solution, and (*d*) the cyclopentanone ring is not required for photochemical activity. We are now carrying out quantitative work to determine the mechanism of the photochemical reactions of chlorophyllin.

[1] Willstätter, R., and Stoll, A., *Untersuchungen über Chlorophyll* (Springer, Berlin, 1913). West, C. J., *Biochem. Bull.*, **3**, 229 (1914).
[2] Oster, G., *J. Chim. Phys.*, **55**, 899 (1958).
[3] Oster, G., *J. Polymer Sci.*, **16**, 235 (1955).
[4] Oster, G., and Bellin, J. S., *J. Amer. Chem. Soc.*, **79**, 294 (1957).
[5] Bellin, J. S., and Oster, G., *J. Amer. Chem. Soc.*, **79**, 2461 (1957).
[6] Jacobs, E. E., Vatter, A. E., and Holt, A. S., *Arch. Biochem. Biophys.*, **53**, 228 (1954).
[7] Falk, J. E., and Willis, J. B., *Austral. J. Sci. Res.*, **4**, 579 (1951).
[8] Sidorov, A. M., and Terenin, A. N., *Optika i Spektro.*, **8**, 254 (1960).
[9] Fischer, H., and Pfeiffer, H., *Liebigs Ann. Chem.*, **555**, 94 (1944); Holt, A. S., and Jacobs, E. E., *Amer. J. Bot.*, **41**, 710 (1954).
[10] Oster, G., Bellin, J. S., Kimball, R. W., Schrader, M. E., *J. Amer. Chem. Soc.*, **81**, 5095 (1959).
[11] Böhi, J., *Helv. Chim. Acta*, **12**, 121 (1929). Ghosh, J. C., and Sen Gupta, S. B., *J. Ind. Chem. Soc.*, **11**, 65 (1934). Livingston, R., and Pariser, R., *J. Amer. Chem. Soc.*, **70**, 1510 (1948).
[12] Compare, Ichimura, S., and Rabinowitch, E., *Science*, **131**, 1314 (1960).
[13] Oster, G., and Wotherspoon, N., *J. Amer. Chem. Soc.*, **79**, 4836 (1957).
[14] Krasnovsky, A. A., *Doklad. Akad. Nauk SSSR*, **60**, 421 (1948).
[15] Bannister, T. T., *Plant Physiol.*, **4**, 246 (1959).
[16] Compare Rachow, B., and König, H., *Z. Elektrochem.*, **62**, 482 (1958) and Krasnovsky, A. A., *Uspekh. Khim.*, **29**, 344 (1960).
[17] Rabinowitch, E., Jacobs, E. E., Holt, A. S., and Kromhout, R. A., *Z. Physik*, **133**, 261 (1952).
[18] Brody, S. S., and Brody, M., *Nature*, **189**, 547 (1961).
[19] Rabinowitch, E. I., *Photosynthesis and Related Processes*, Chapters 18, 35 (Intersci. Publishers, New York, 1945 and 1956). Livingston, R., *Radiation Res. Suppl.*, **2**, 196 (1960) and *Quart. Revs.*, **14**, 174 (1960).

A QUANTITATIVE THEORY OF THE CHARACTER OF CHEMICAL BONDING IN BINARY COMPOUNDS

By Prof. EDWARD GÖRLICH

Department of Silicate Chemistry, School of Mining and Metallurgy, College of Ceramics, Cracow

In view of the great difficulties encountered in the case of a strict quantum-mechanical treatment of many chemical problems, especially in the field of inorganic chemistry, many attempts were aimed at a more qualitative approach to the problems involved by other, much simpler methods. Of such, the most widely known are Pauling's theory of electronegativity, the theory of oxygen attraction, first put forward by van Arkel and de Boer, Fajans' theory of ionic polarization and his quanticule theory and Weyl's theory of screening. It appears to me that, accepting as a first approximation a purely electrostatic character of chemical bonding, it is possible to establish a quantitative theory of binary chemical compounds.

The proposed theory is based on the following assumptions:

(1) Every kind of chemical compound (salts, metals, molecular compounds) is made of cations, or positive cores equivalent to cations, and a number of valency (or bonding) electrons, which form various configurations of electron clouds.

(2) Homonuclear compounds (pure elements) are built of one kind of cation or positive core only. Differences in their chemical characteristics are due to the different degrees of polarization of electron

clouds by the specific cations or cores. If there is no perceptible effect of polarization a purely metallic bonding results. A growing effect of polarization results in a more and more atomic (covalent) character of binding.

(3) The ionic character of bonding in binary heteronuclear compounds is due to the asymmetry of the attraction of the valency electrons by the two different kinds of cations or positive cores present, one of which is pulling the electrons more strongly than the other. In a purely ionic binary compound the strength of the field of one cation is completely negligible in comparison with the other which, in consequence, forms a positive core of an anion.

The proposed system may be presented in the form of a diagram with the following co-ordinates:

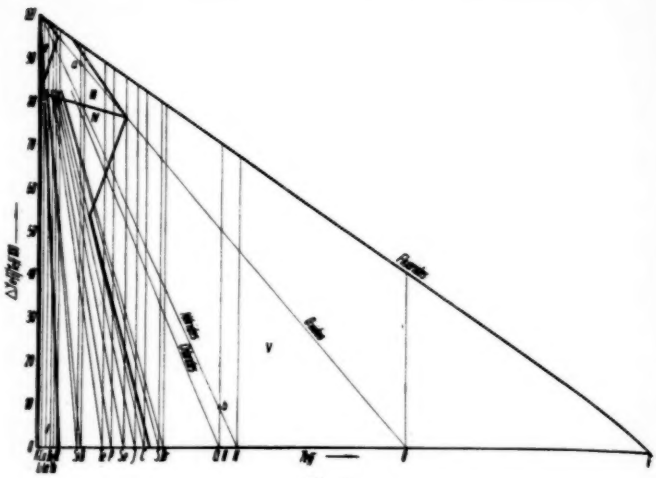


Fig. 1

(*a*) The values of the ionic potential φ, which, according to Cartledge (1926), is a measure of the polarizing ability of cations, are taken as abscissæ. The abscissa is the axis of homonuclear compounds, along which the ionic potential φ increases from zero (the origin of the co-ordinate system) corresponding to a purely metallic bond up to the value of 87·5 for F^{7+}, corresponding to an extremely atomic (covalent) character of bonding in F_2.

(*b*) As the ordinate, the relative values of the differences between the ionic potentials of cations or positive cores, expressed in percentage of the ionic potential $\varphi_1 (\varphi_1 > \varphi_2)$, may be accepted: $\frac{\Delta\varphi}{\varphi_1} \times 100$.

The value of this parameter is zero for a purely metallic bond (taken as the origin of the system) and for purely ionic bonds it reaches the value of 100.

The diagram for the proposed system of bond character of binary compounds may be constructed in the form of a right-angled triangle with apexes representing purely metallic, ionic and most nearly atomic (covalent) character of bonding (Fig. 1).

The values of ionic radii given by Ahrens[1] were accepted in computations of ionic potentials throughout this article.

It is, however, more correct to use instead of 'formal' ionic potentials, as formerly proposed by Cartledge, the effective (actual) values of ionic potentials. The effective ionic potential φ_{eff} is related to the electronegativity as proposed by Pauling (log φ_{eff} is a linear function of Pauling's electronegativity). It constitutes also a measure of the acidity (or basicity) of the cation[2]:

$$\frac{\varphi_{eff}}{\varphi} = x$$

For noble gas-type cations with the same charge, the coefficient x has the same constant value. As a measure of the actual field strength of cations the values of the so-called field function $F = I^p/r$ are proposed here[1], where I^p is the pth ionization potential with charge p of the chemical element forming a given cation) and r is the ionic radius. As may be demonstrated, the relation $F = I^p/r = x \times \varphi - a$ is strictly valid for all inert gas-type cations of the same charge. Putting $I^p/r + a = \varphi_{eff}$ we can find the value of the coefficient x (Table 1). Only inert gas-type cations are considered here; the more complex problems of the elements of the sub-groups of the periodic system will be treated in a separate paper.

The character of bonding of a binary compound is represented by a point on the diagram (Fig. 1), lying at the intersection of two lines, one of them parallel to the axis of the ordinate ($M - I$) and cutting the axis of the abscissa at φ_{eff} of the weaker cation or core (that is, one which has the smaller value of φ_{eff}) and the second connecting the value φ_{eff} of the stronger cation or core (that is, one which has the greater value of φ_{eff}) with the ionic apex of the triangle. For example, point *a* represents the character of the bond Si—O, and point *b* that of N—Cl.

As Fig. 1 shows, all points representing fluorides lie on the same line connecting the φ_{eff} value for

Table 1. Values of the Coefficients x* of Cations or Positive Cores of Inert Gas-type with the Charge z

z	x
1	0·65
2	1·08
3	1·50
4	1·90
5	2·30
6	2·70
7	3·10
8	3·50

* The coefficient $x = \varphi_{eff}/\varphi$ may be regarded as a relative electrostatic electronegativity value of the cations or positive cores.

Table 2

Field* (Figs. 1 and 2)	Type of binary compound	Character of electrostatic bonding	State of aggregation†
I	Metals and intermetallic compounds	Metallic	Solid
II	Salts (alkaline oxides included)	Ionic	Solid
III	Polymers	Ionoidic (sub-ionic)	Solid (glass-forming)
IV	Sub-metals (semiconductors and semimetals)	Sub-metallic (metalloidic)	Solid
V	Molecular compounds	Atomic (covalent)	Solid, liquid or gas
VI	Inert gases	None	Gas (free atoms)

* Many of the properties of the binary compounds, resulting from the different nature of their chemical bonds, are, of course, changing more gradually than is suggested by the sharp boundary lines shown on Figs. 1 and 2.

† At room temperature and pressure.

F^{7+} with the apex I, and also points for all other compounds with common anion, namely, oxides, sulphides, chlorides, nitrides, etc., lie on their respective lines.

Various physical and chemical properties of binary compounds (as, for example, their structures) are reasonably ordered by the proposed system. It enables a classification of binary chemical compounds (Table 2).

Some irregularities noticeable among the halides are due to the fact that the proposed diagram (Fig. 1) is a projection of a space diagram with n as the third axis of co-ordinates (n being the period of the Mendeleyevian system to which the given element belongs). The diagrem $\log n - \log \varphi_{eff}$ ordering the homonuclear compounds (pure elements) will be presented in a later communication.

It is to be noted that for the elements of the main groups of the periodic system $\log \varphi_{eff}$ is a linear function of $\log z$, z being the group of the periodic system. It is also to be mentioned that the expression $\Delta\varphi_{eff}/\varphi_{eff_1}$, where $\varphi_{eff_1} > \varphi_{eff_2}$, may be considered as a measure of the chemical affinity of the elements forming a heteronuclear compound. It was used by me to systematize the binary systems of equilibria for compounds with common anions[3].

The projection points on the diagram given by Fig. 1 are very unevenly distributed. To obtain a more uniform dispersion of points we must resort to a logarithmic scale. This may be done by accepting the value φ_{eff} for Fr^+ as unity ($\varphi'_{eff}Fr = 1{,}000$). This choice is justified by the fact that francium, as a homonuclear compound, must have the most nearly pure metallic character of bonding among all known elements. As the others (atomic or covalent) we take F^{7+}, because fluorine in the F_2 molecule forms the most nearly atomic bonds known. Putting $\log \varphi'_{eff}$ value for Fr (that is, zero) at the origin of the system of co-ordinates (point M) and accepting the $\log \varphi'_{eff}$ value for F (2·876) as 100 (point A), we obtain a relative logarithmic scale on which the atomic character of bonding in homonuclear binary compounds may be expressed in percentages of that found in the F_2 molecules. The diagram $I - M - A$ may now be presented in the convenient form of an equilateral triangle (Fig. 2). The ionic character of

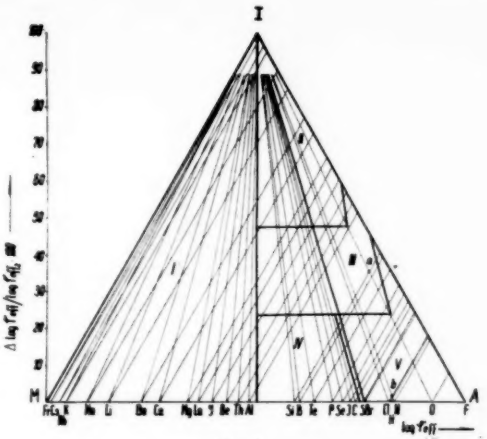


Fig. 2

bonding is here also expressed in percentages: $\Delta \log \varphi'_{eff}/\log \varphi'_{eff_1} \times 100$. The ionic character of bonds in Fr—F has on the absolute scale (Fig. 1) the value 99·87 per cent in comparison with 100 per cent on the relative logarithmic scale (Fig. 2). The bond Si—O in SiO_2 is 35·8 per cent ionic on this scale (point a, Fig. 2). The vertical line on this diagram is only an auxiliary scale.

All points for fluorides, oxides and so on lie on the respective lines connecting the values for F, O and so on, lying on the M–A axis with the I—apex of the triangle—as on the diagram Fig. 1. The fields bear the same denominations as on the previous diagram. The proposed systems are valid for room temperature and atmospheric pressure. Possible defects in solids and the dispersion energy share in bonding are not taken into account here, nor are the magnetic forces.

Sincere thanks are due to Prof. A. Bielański for valuable discussions.

[1] Ahrens, L. H., *Geochim. Cosmochim. Acta*, **3**, 5 (1953).
[2] Görlich, E., and Stopka, Z., *Zeszyty Naukowe AGH, Ceramika*, **1**, 19 (1956).
[3] Görlich, E., *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, **8**, 231 (1960).

CHITINASE: AN ADDITION TO THE LIST OF HYDROLASES IN THE DIGESTIVE TRACT OF VERTEBRATES

By Dr. CHARLES JEUNIAUX
Department of Biochemistry, University of Liège

It was thought for a long time that, in animals, chitinases were present in snails only. The distribution of these enzymes among invertebrates has appeared to be much more extensive. They have been found by Tracey in protozoa[1], in nematodes[2], and in earthworms[3], and by me in arthropods[4–6], cœlenterates[7], polychæte worms[7], etc. The presence of chitinolytic enzymes among vertebrates has, however, never been suspected. The enzymic lysis of chitin is known to be actually performed by two different enzymes, acting consecutively[8–9]: a chitinase (mucopolysaccharidase) and a chitobiase (β-acetylglucosaminidase). This 'chitinolytic system' and these two enzymes have been searched for in the digestive juices and in aqueous extracts of digestive glands and digestive epithelia of some fishes, lizards, birds and mammals.

In order to detect the chitinolytic system (chitinase + chitobiase), the enzymic extracts were incubated with a chitin suspension (from *Sepia* pen) at 37° C. and pH 5·2 for 3 hr.; the acetylglucosamine liberated was measured by a method described by Reissig *et al.*[10]. Controls without enzyme or without substrate were run simultaneously.

In order to detect chitinase in enzymic extracts containing little or no chitobiase, the activity of these extracts on chitin has also been investigated after addition of an excess of chitobiase in the enzy-

Table 1. Chitinolytic enzymes in digestive juices of some Vertebrates

Species	Digestive juices or contents	Activity of chitinolytic system: acetylglucosamine liberated, μgm./hr./ml.
Carassius auratus L.	Anterior intestine	113
	Posterior intestine	814
Gasterosteus aculeatus L.	Whole digestive contents	872
Lacerta viridis Laur.	Gastric juice	1,200
Testudo hermanni J. F. Gmelin	Gastric juice	0
	Intestinal contents	0
Passer domesticus L.	Gastric juice	180
Turdus merula L.	Gastric juice	1,550
Oryctolagus cuniculus L.	Gastric contents	0
	Intestinal contents	2·5*
	Cæcal contents	0

* Non-significant result.

Table 2. Distribution and localization of chitinase and chitobiase in digestive glands and tissues of some vertebrates

Species	Organs	Activity = acetylglucosamine liberated, μgm./hr./gm. fresh tissues		
		Chitinolytic system*	Chitinase†	Chitobiase‡
Carassius auratus L.	Mucosa anterior intestine	43–88	490	440
	Mucosa posterior intestine	288	—	72
	Pancreas (+ Liver)	—	67	62
Lacerta viridis Laur.	Gastric mucosa	1437–1570	8,064	250–740
	Intestine	0–3·3§	—	45
	Pancreas	810–1350	13,120	0–160
Testudo hermanni J. M. Gmelin	Gastric mucosa	0	0	45
	Intestinal mucosa	0	0	57
Turdus merula L.	Mucosa of glandular stomach	340	3,114	89
	Intestine (first third)	26	144	56
	Pancreas	13§	13§	0
Rhinolophus ferrum-equinum Schreb.	Gastric mucosa	347	5,280	71
	Intestine	0	12§	23
	Pancreas	0	0	29
Oryctolagus cuniculus L.	Gastric mucosa	0	0	0
	Intestine	0	0	70
	Pancreas	0	7·8§	72

* Liberation of acetylglucosamine from chitin, by the enzymic extract.
† Liberation of acetylglucosamine from chitin, by the enzymic extract after addition of an excess of chitobiase.
‡ Liberation of acetylglucosamine from 'depolymerized chitin', a preparation containing chitobiose and other small polymers of acetylglucosamine.
§ Non-significant results.

mic test. A commercial preparation of β-glucosidase (Nutritional Biochemicals Corporation) provided a convenient source of chitobiase, free from chitinase. In these cases, the presence of chitinase was also confirmed by the nephelometric method[11], which does not depend on the presence or absence of chitobiase[9]. In order to measure the chitobiase, the liberation of acetylglucosamine has been followed, using a preparation of 'depolymerized chitin' as substrate[12] (a preparation which contains chitobiose and other small polymers of acetylglucosamine).

The results are summarized in Tables 1 and 2. Chitinolytic enzymes have been found in the digestive tract of six species of vertebrates, among the eight species so far investigated, the digestive juices and tissues of turtle and rabbit being entirely devoid of chitinases. In all these eight species, muscles, kidney and liver extracts were found to be free from chitinases, the presence of chitobiase only being evident in the livers of *Lacerta* and *Testudo*.

The following tentative conclusions may be drawn from these results:

(1) The chitinolytic enzymes have been found not only in the digestive contents, but also in glands and in carefully washed gastric or intestinal mucosa. Such localization does not support the hypothesis of a bacterial origin.

(2) All the species where chitinase has been found are insectivorous, or eat chitin-covered preys, at least occasionally. In contrast, the turtle and the rabbit, which do not possess any trace of chitinase secretion, are typical phytophagous animals. This observation, if confirmed for other species, would represent the only clear-cut case, with that of lactase, of a correlation between diet and digestive enzymes among vertebrates.

(3) The site of chitinase secretion seems to show an evolution towards a higher specialization of tissues: in the goldfish, the secretion of chitinase is a property of the whole epithelium and of pancreatic islets. This faculty is restricted to the gastric mucosa and the pancreas in *Lacerta*: in bats, the gastric mucosa alone has this property.

In contrast, the secretion of chitobiase, although often very weak, occurs in the majority of tissues, even in the turtle and in the rabbit.

(4) The cases of the pancreas of *Lacerta* and of the gastric mucosa of *Turdus* and of *Rhinolophus* are the first known examples of tissues being able to synthesize large amounts of chitinase, but secreting only very small amounts of chitobiase.

1 Tracey, M. V., *Nature*, **175**, 815 (1955).
2 Tracey, M. V., *Second Intern. Cong. Biochem.*, Paris, **242** (1952).
3 Tracey, M. V., *Nature*, **167**, 776 (1951).
4 Jeuniaux, Ch., *Mém. Soc. Roy. Entom. Belg.*, **27**, 312 (1955).
5 Jeuniaux, Ch., *Arch. Intern. Physiol. Biochem.*, **64**, 583 (1956)
6 Jeuniaux, Ch., *Arch. Intern. Physiol. Biochem.*, **68**, 684 (1960).
7 Jeuniaux, Ch., *Cahiers Biol. Marine* (in the press).
8 Zechmeister, L., and Toth, G., *Enzymologia*, **7**, 165 (1939).
9 Jeuniaux, Ch., *Arch. Intern. Physiol. Biochem.*, **67**, 115 (1959).
10 Reissig, J. L., Strominger, J. L., and Leloir, L. F., *J. Biol. Chem.*, **217**, 959 (1955).
11 Jeuniaux, Ch., *Arch. Intern. Physiol. Biochem.*, **66**, 408 (1958).
12 Jeuniaux, Ch., and Devigne, J., *Arch. Intern. Physiol. Biochem.*, **68**, 411 (1960).

A PRECIPITATING ANTIBODY TO INSULIN

By VALERIE E. JONES and Prof. A. C. CUNLIFFE

Department of Bacteriology, King's College Hospital Medical School, London, S.E.5

ANTIBODIES to insulin preparations have been detected by many of the conventional immunological methods in the sera of insulin-treated diabetic and mental patients, and of animals immunized with insulin, but convincing demonstrations of antibodies to insulin itself are few[1]. Furthermore, Lapresle and Grabar[2] have directed attention to the presence of precipitating antibodies in the sera of some diabetics, to antigens other than insulin. These antigens were found in the mother liquor which remains after the initial crystallization of insulin. Precipitating antibodies to insulin were found by Moloney and Aprile[3], in a horse hyperimmunized with crystalline insulin, and both insulin and the antibody were recovered from the specific precipitate. They were, however, unable to find precipitating antibodies in several other species, including guinea pigs, which were immunized in the

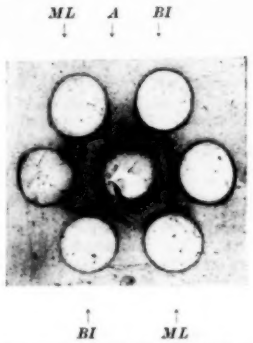


Fig. 1. Precipitation of guinea pig anti-bovine insulin serum: *A* (centre of figure) and some insulin preparations in agar gel; Boots bovine insulin, *BI*, Wellcome pig insulin, *PI*, and mother liquor, *ML*

same manner. Berson and Yalow[4] have attributed their failure to find precipitating antibodies in the sera of insulin-treated diabetics to the univalence of insulin. Nevertheless, we have found that guinea pigs readily produce precipitating antibodies when immunized with large doses of crystalline insulin preparations, and we present here evidence that these are antibodies to insulin.

Bovine insulin, crystallized six times (Boots, Ltd.), was dissolved in 0·002 *N* hydrochloric acid and carefully emulsified with Freund's 'Complete Adjuvant' (Difco) to give a final insulin concentration of 1·5 mgm./ml. Adult guinea pigs each received 1·0 ml., injected beneath the skin and distributed between three or four sites. After 4 weeks a further 1·0 ml. was similarly injected and one month later precipitating antibodies to the insulin preparation could be demonstrated in the serum. Thirty immunized guinea pigs all produced this type of antibody.

Precipitation of the antigen–antibody complex was first detected by the gel diffusion technique of Ouchterlony[5] (Fig. 1). Solutions containing 200 μgm./ml. of several-times crystallized bovine and pig insulins were placed in the outer wells as was a solution containing 200 μgm. per ml. of mother liquor solids from an initial crystallization (kindly provided by Dr. Lapresle, Paris). This last material contained only 16–18 per cent of the concentration of insulin present in the other wells. The guinea pig antiserum in the centre well, although forming a single identical precipitation band with the crystalline insulins, gave no visible reaction with the mother liquor.

Specific precipitates of antigen and antibody were measured quantitatively[6], using 1·0 ml. amounts of two pools of guinea pig antiserum (*A* and *B*) and varying amounts of two different bovine insulin preparations, crystallized six times (Boots, Ltd., 22·2 units/mgm. and Burroughs Wellcome and Co., 25 units/mgm.). Mixtures of antiserum and antigen were incubated for 4 hr. at 37° C. and for a further two days at 4° C. The precipitates were washed three times in 2 ml. of saline and then dissolved in 2·5 ml. of 0·1 *N* sodium hydroxide. Total protein was determined from the E_{280}, measured by means of a 'Uvispek' spectrophotometer (Hilger and Watts) with a 1-cm. quartz cell. A factor of 0·715, estimated for guinea pig gamma globulin in 0·1 *N* sodium hydroxide, was used to convert the E_{280} to mgm. protein/ml. (Fig. 2). At the point of maximum precipitation, pool *B* antiserum gave 1·36 mgm. of specific precipitate with 75 μgm. of Boots insulin; pool *A* gave 1·00 mgm. with 50 μgm. of this insulin preparation but only 0·82 mgm. with the Wellcome preparation. Assuming that all the antigen was precipitated at the point of maximum precipitation, the ratio of antibody to antigen at this point varies between 15·5 and 19. A single curve using pool *A* and once-crystallized pig insulin (Wellcome) is also shown (Fig. 2). The smaller quantity of specific precipitate formed with this heterologous insulin preparation suggests a species specificity which is interesting in view of the finding of Berson and Yalow[7] that bovine insulin is more firmly bound to human 'antibovine-pig insulin' serum than is pig insulin, and also accords with the difference in chemical composition of pig and bovine insulins[8].

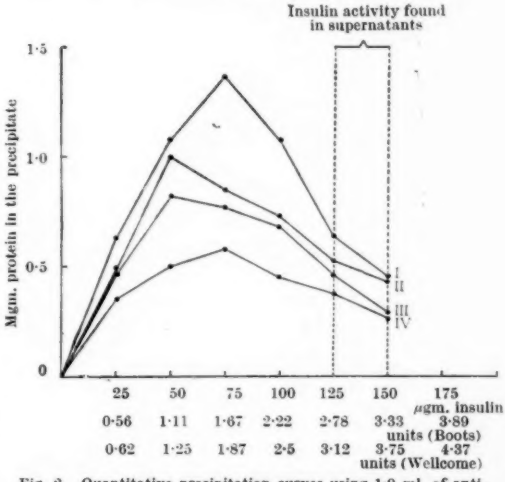


Fig. 2. Quantitative precipitation curves using 1·0 ml. of antiserum. Curve I, pool *B* + bovine insulin, and curve II, pool *A* + bovine insulin (Boots, 22·2 units/mgm.); curve III, pool *A* + bovine insulin, and curve IV, pool *A* + pig insulin (Wellcome, 25 units/mgm.)

The supernatants obtained after removing the specific precipitates were tested for free antigen and antibody. In relatively large antigen excess, insulin activity was detected by the mouse convulsion test (Fig. 2) and both antigen and antibody were detectable in great antigen and antibody excess respectively by precipitation in agar gel. Both these methods, however, are relatively insensitive and incapable of detecting amounts smaller than 0·1 unit (4·5 μgm.) of insulin in each supernatant.

Precipitation, at the point of maximum precipitation, was probably not complete, since, in several experiments with longer incubation periods, somewhat larger specific precipitates were obtained. For example, with an incubation period of 7 hr. at 37° C., 1·0 ml. of pool *B* and 75 μgm. of insulin (Boots) gave 1·72 mgm. of specific precipitate, corresponding to an antibody–antigen ratio of 22. Attempts were made to check the completeness of precipitation using iodine-131 labelled insulin with a specific activity of less than 1 mc./mgm. (kindly prepared by Dr. Samols). Larger amounts of the labelled than of unlabelled antigen were needed to produce maximum precipitation, suggesting that some denaturation of the insulin had occurred. Because of this, results were not entirely conclusive; however, 47 per cent of the total radioactivity added was recovered in precipitates which contained only 66 per cent of the total precipitable antigen–antibody protein found when unlabelled insulin was used.

An attempt was made, using gel diffusion methods devised by Allison and Humphrey[9], to estimate the size of the antigen molecules which initiate this precipitation. The diffusion coefficient was estimated to be 9·6, assuming that the diffusion coefficient of guinea pig antibody is 3·8. The 'molecular sieve' effect of gelatin gels was also used and the antigen diffused through 38 per cent gelatin. Both methods indicate that the molecular weight is less than 24,000.

These preliminary results suggest that the precipitating guinea pig antibody is indeed antibody to insulin and not to an impurity in the preparations. If this is so, it can be concluded from the results of the experiments designed to estimate molecular size of the antigen, that the insulin antigen is mostly in the aggregated form.

We are grateful to Dr. E. Samols, Royal Free Hospital, London, for preparing the labelled insulin. This work is supported by grants from the British Diabetic Association and the Joint Research Committee of King's College Hospital and Medical School.

[1] Wright, P. H., *Brit. Med. Bull.*, **16**, 219 (1960).
[2] Lapresle, C., and Grabar, P., *Rev. Franç. d'Études Clin. Biol.*, **11**, 1025 (1957).
[3] Moloney, P. J., and Aprile, M. A., *Canad. J. Biochem. Physiol.*, **37**, 793 (1959).
[4] Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **38**, 1996 (1959).
[5] Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, **32**, 231 (1953).
[6] Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry* (1948).
[7] Berson, S. A., and Yalow, R. S., *J. Clin. Invest.*, **38**, 2017 (1959).
[8] Brown, H., Sanger, F., and Kitai, R., *Biochem. J.*, **60**, 556 (1955).
[9] Allison, A. C., and Humphrey, J. H., *Immunology*, **3**, 95 (1960).

ANTIBIOTIC PROPERTIES OF MYCELIUM AND METABOLITES OF *MYROTHECIUM RORIDUM* TODE

By ANDRZEJ NESPIAK, MARIAN KOCÓR and ANTONI SIEWIŃSKI

Departments of Phytopathology and General Chemistry, College of Agriculture, Wroclaw

THE fungus *Myrothecium roridum*, belonging to the Fungi Imperfecti, group Moniliales[1], is known as a parasite of leaves of *Gardenia*, tomatoes, violets, kidney beans and some other plants[2,3]. It has been also found in soil[4] and in the dead plant tissues. A detailed morphological analysis of species of *Myrothecium roridum* and related *Myrothecium verrucaria* and *Myrothecium inundatum* was undertaken by White and Downing[5] on the basis of observations of several strains. They confirmed the great morphological similarity between *Myrothecium roridum* and *Myrothecium verrucaria*, and also found that these two species differed only in their manner of decomposition of cellulose, and their occurrence. They also demonstrated the complete identity of *Myrothecium roridum* with a species known as *Metarrhizium glutinosum* Pope, which produced, according to Brian and McGowan[6], a new metabolite, glutinosine.

The scarcity of data on metabolite production by *Myrothecium roridum*[7,8] directed our attention to this problem, and now we present some observations on the activity of the mycelium and the metabolites of *Myrothecium roridum* towards some fungi pathogenic for cultivated plants.

The strain of *Myrothecium roridum* isolated by one of us[9], and used for further experiments, has been characterized by formation on glucose-potato-agar of a dense, white mycelium with concentric black sporodochia. The smoky, light olive-green conidia (8–10μ long, 2–4μ thick) form black, slime masses, consisting of cylindrical or spindle-shaped cells containing oil drops. The fungus grows and sporulates very easily both on malt-agar and on malt liquid (20 gm. malto-extract produced by Spółdzielnia Pracy 'Warta' Śrem (Poland) in one litre distilled water), in the latter case the concentration of sporodochia not being easily visible.

For the biological tests of fungicidal activity of *Myrothecium roridum* the following species of fungi were used: *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium equiseti*, *Fusarium anguioides*, *Fusarium* sp., *Mucor* sp., *Alternaria*

Table 1. Inhibiting Effect of the Mycelium of *Myrothecium roridum*, and its Metabolites on the Growth of some Fungi

Species	Mycelium		Metabolic solution						Metabolites			
	malt-agar	potato-agar	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	control	myrothecin	minor metabolite of m.p. 204–205°C.	mother liquor	control
Penicillium notatum	++	++	++	++	+	+	++	+	++	++	+++	—
Aspergillus fumigatus	+	++	++	+	+	+	++	+	+	+	++	—
Aspergillus niger	++	++	++	+	+	+	+	+	—	+	++	—
Fusarium equiseti	+	+	+	+	+	+	+	+	—	—	+	—
Fusarium sp.	+	+	+++	+	+	+	++	+	—	+	++	—
Fusarium anguioides	++	++	+++	+++	+	+	+	+	—	—	++	—
Verticillium albo-atrum	+++	+++	+++	+++	+	+	+++	+	+	++	+++	—
Phoma betae	+++	+++	+++	++	+	+	++	+	+	++	+++	—
Botrytis cinerea	+++	+++	+++	+++	+	+	+++	+	+	++	+++	—
Mucor sp.	—	—	+*	+	+	+	+	+	—	—	+	—
Rhizopus nigricans	—	—	+*	+	+	+	+	+	—	—	—	—
Trichothecium roseum	+	+	++	++	+	+	+	+	—	+	++	—
Alternaria tenuis	++	++	++	+	+	+	++	+	—	+	++	—
Hormodendrum sp.	+	+	++	++	+	+	++	+	+	+	++	—
Cephalosporium roseum	++	++	++	++	+	+	++	+	—	—	++	—

+++, Zone of inhibition more than 10 mm. or insignificant growth of mycelium for exp. 2 *a b c d e*
++, „ „ „ between 5 and 10 mm. or average „ „ „ „ „ „
+, „ „ „ between 0 and 10 mm. or normal „ „ „ „ „ „
—, No inhibition
* Only weak sporulation observed.

tenuis, *Verticillium albo-atrum*, *Botrytis cinerea*, *Phoma betae*, *Cephalosporium roseum*, *Trichothecium roseum*, *Hormodendrum* sp., *Rhizopus nigricans*.

Analysis of the metabolic solution of *Myrothecium roridum* in the usual way, that is, extraction with chloroform, chromatography on activated alumina and crystallization, yielded one major and two minor crystalline metabolites. The major metabolite, which we called myrothecin, has a molecular weight of 334, and on this basis and on its elemental analysis it was assigned the formula $C_{19}H_{22}O_6$. The substance does not melt at temperatures up to 350° C. and has $[\alpha]^{20}D = +174°$ (in chloroform).

The second metabolite has a melting point of 204–205°, the third one 115–120°. It must be stated that these compounds could be isolated only when *Myrothecium roridum* was grown on malt liquid, otherwise an entirely different production of metabolites occurred.

The biological tests were carried out in the following manner :

(1) The mould *Myrothecium roridum* was grown in Petri dishes on potato- or malt-agar, and simultaneously, or after 4 days, the species investigated were inoculated at distances of 4 cm. The zones of inhibition were measured in millimetres. (2) The crude filtrates of the metabolic solution of 4-week-old cultures of *Myrothecium roridum* were investigated for their fungicidal activity in the following way : filtrates were used for the preparation of the 5 following media, on which the test fungi were grown :

(*a*) 1 litre filtrate + 20 gm. agar ; (*b*) 0·5 litre filtrate + 0·5 litre malt liquid + 20 gm. agar ; (*c*) 1 litre filtrate passed through active charcoal + 20 gm. agar ; (*d*) the same filtrate + 0·5 litre malt liquid + 20 gm. agar ; (*e*) dried mycelium from 4-week-old cultures of *Myrothecium roridum* suspended in 1 litre distilled water + 20 gm. agar, and (*f*) control medium prepared from malt liquid + 20 gm. agar.

(3) The chloroform extracts of the metabolic solution of *Myrothecium roridum* were investigated by means of the paper-disk plate method. Approximately 10 mgm. dried chloroform extract (or crystals) were dissolved in chloroform, and 20 filter paper disks were saturated with this solution. After drying, each disk was moistened with water and put on a malt-agar medium, into which the suspension of spores of the test fungus was inoculated. The zones of inhibition were measured in millimetres.

The results are presented in Table 1 : they show that :

(1) The growing mycelium of *Myrothecium roridum* inhibits strongly the growth of the majority of test fungi except the quickly developing species. (2) The metabolic solution of *Myrothecium roridum* also has a strong inhibiting effect, when not treated with charcoal, which adsorbs the fungicidal metabolites. The suspension of dried mycelium is not very active. (3) The major metabolite (myrothecin) is not very active, whereas the first minor metabolite (melting point 205° C.) and the non-crystalline mother liquors are highly active. (4) *Myrothecium roridum* does not produce glutinosine, but a number of crystalline compounds (at least three) and possesses high fungicidal activity.

[1] Gilman, J. C., *Manual of Soil Fungi* (London, 1959).
[2] Fergus, Ch. L., *Mycologia*, **49**, (6). 124 (1957).
[3] Thirumalachar, M. J., and Mishra, J. N., *Rev. App. Mycol.*, **33**, 338 (1954).
[4] Atkinson, R. G., *Rev. App. Mycol.*, **34**, 326 (1955).
[5] White, W. L., and Downing, M. H., *Mycologia*, **39** (5), 546 (1947)
[6] Brian, P. W., and McGowan, J. C., *Nature*, **157**, 334 (1946).
[7] Brian, P. W., Hemming, H. G., and Jefferys, E. G., *Mycologia*, **40** (3), 363 (1948).
[8] Brian, P. W., *Bot. Rev.*, **16** (6), 357 (1951).
[9] Nespiak, A., and Vörös, J., *Agrokémia és Talajtan*, **9** (4), 145 (1960).

EFFECT OF WHOLE-BODY IRRADIATION AND CORTISONE ON THE DEVELOPMENT OF *OSTERTAGIA* SPP. IN SHEEP

By J. D. DUNSMORE

Department of Veterinary Pathology and Bacteriology, University of Sydney

Effect of Whole-Body Irradiation on Antibody Response of Sheep

IN the course of experimental work on the life-cycle of sheep helminths it became necessary to utilize experimental methods of suppressing the sheep's antibody response. One method of accomplishing this is by whole-body irradiation ; in view of the lack of available data on response of the animal to whole-body irradiation the following is presented to indicate the results that I have obtained.

Information available (*Handbook of Biological Data*, W. B. Saunders Co.) indicated that the LD_{50} (30 day) for goats, the species nearest sheep for which data were available, was 350 rads. It also appeared that the dose necessary to suppress antibody response is about 80 per cent of the LD_{50}. Hence the initial test dose was approximately 300 rads. The irradiation source used was 310 c. cobalt-60, to which the sheep was exposed at a distance of 160 cm. Half the exposure was delivered to each side of the sheep. Total exposure was 4·5 hr. to deliver 300 rads. The test antigen used was a formalin-killed suspension of *Salmonella typhimurium* injected 36 hr. and 5 days following irradiation. This had been shown to induce marked agglutinin production in normal sheep. In a sheep exposed to 300 rads only a slight reduction in antibody response was noted although the leucocyte count dropped to a minimum (48 hr. post-irradiation) of 960 per mm.^{3} with only 2 per cent lymphocytes in the differential count.

Following this initial test two sheep were each exposed to 440 rads (6 hr. exposure, 160 cm. from 310 c. cobalt-60) and subjected to similar antigenic stimulus. No antibody was detected by orthodox agglutination techniques up to 19 days following irradiation in the first sheep. The second sheep died suddenly 14 days after irradiation without having developed any detectable antibody to the *Salmonella* antigen. At autopsy there was evidence of septicæmia and a Gram-negative bacillus was cultured from heart blood, but not identified.

These results indicated that 440 rads, applied equally to both sides of the sheep, suppressed antibody production as assessed by the response to a formalin-killed *Salmonella typhimurium* antigen.

Following these preliminary experiments, four sheep have been irradiated at the above dose. In all cases the hæmatological picture indicated a very severe depletion of body lymphocytes with total white cells dropping in all cases to less than 20 per cent of the pre-irradiation figure, the minimum being reached 48 or 72 hr. following irradiation. The lymphocytes were the cells most affected initially, dropping to less than 2 per cent in all cases within 72 hr. and then rising again during the next two weeks. The granulocytes reached a minimum 7–10 days after irradiation so that at this stage the total count was sub-normal, of which about 80–90 per cent were lymphocytes. From the experimental results obtained in this work on the role of immunity to developing helminths it did appear that the host immunological response had been depressed. The nature of the experiment prevented any attempt to confirm this serologically.

In all the sheep a febrile reaction was noted which became severe (temperature exceeding 106°F.) in two animals. The experiment did not allow antibiotic therapy so that these sheep were killed and autopsied 13 and 14 days after irradiation. The remaining two sheep survived the full 15-day experimental period with little evidence of harm. The two sheep severely affected both showed septicæmia with severe gastro-enteritis, but again the Gram-negative bacillus isolated did not fit a readily identifiable bacterial group. Splenic and lymphoid tissue in all sheep was markedly atrophied and bone marrow smears showed severe agranulocytosis. The hæmatological picture had showed that the early lymphocytopenia was later replaced by a relative lymphocytosis as the total granulocyte count became depressed (7–10 days following irradiation). In two sheep that survived irradiation and experimentation the hæmatological picture took 12 weeks to return to normal.

It appears that 440 rads whole-body irradiation will largely suppress the antibody response of sheep, but the sheep is likely to succumb to bacterial infection unless protected by antibiotics.

Effect of Cortisone and Whole-Body Irradiation on Development of *Ostertagia* spp. in Sheep

It has been shown previously[1] that the development of a large percentage of the parasites recovered from a sheep following a single large dose of *Ostertagia* spp. larvæ is arrested, and that 14 days following infestation the larvæ are still only in the early 4th stage of development. In an attempt to determine whether this arrested development is due to an immunological response by the host, an experiment was carried out with the object of destroying or reducing the host immunological response by the use of cortisone and by high-level, whole-body irradiation.

In the experiment, 4 groups were used each of 3 sheep which had been raised free of strongyle parasites and were approximately 6 months old. One animal in each group received larvæ only and acted as an untreated control, one was irradiated and one was treated with cortisone. Irradiation at the rate of 440 rads per animal delivered in two equal parts to each side has been found to be effective in largely eliminating agglutinin response to an injected formalized *Salmonella typhimurium* antigen (see previous article). This dose was given 24 hr. prior to larval administration. Hydrocortisone alcohol was injected intramuscularly at a dose of 4 mgm./kgm. body-weight daily commencing 5 days prior to administration of larvæ. 100,000 *Ostertagia* spp. larvæ were injected directly into the rumen, each member of each group receiving larvæ from the same batch.

The sheep were killed 14 days after administration of larvæ and the parasites were collected after thorough washing of the abomasum and peptic digestion of the abomasal wall. The parasites were counted and classified as 'normal' or 'arrested' forms (Table 1). Those recorded as 'arrested' were still in the early 4th stage of development. At least in the untreated control group the unarrested larvæ should properly be classified as 'retarded', these larvæ not having attained the size that can normally by expected of *Ostertagia* spp. if given at a low level of infestation. Thus a population of *Ostertagia* spp. might include some 'arrested' forms (that is, development temporarily ceased in the very early 4th stage), some 'retarded' (the final morphological stages of development have been reached but the size attained is sub-normal) and normal adults of the genus.

Table 1

	Group No.	1	2	3	4	Means
Cortisone treated	Sheep No.	57	54	64	72	
	Total recovered	53,600	31,400	29,150	15,540	32,420
	Number 'arrested'	6,930	1,200	200	2,740	2,770
	Percentage 'arrested'	12·9	3·8	0·7	17·6	8·5
Irradiated	Sheep No.	61	63	55	59	
	Total recovered	43,670	27,520	13,200	43,200	31,900
	Number 'arrested'	13,540	2,860	2,600	4,860	5,960
	Percentage 'arrested'	31·0	10·4	19·7	11·3	18·7
Control	Sheep No.	68	53	56	62	
	Total recovered	50,130	26,000	20,450	24,000	30,140
	Number 'arrested'	16,130	7,340	14,600	14,060	13,030
	Percentage 'arrested'	32·2	28·2	71·4	58·5	43·2

Larval arrest was very much less in cortisone-treated (8·5 per cent) and in the irradiated sheep (18·7 per cent) than in the controls.

Although both cortisone and irradiation cause a variety of effects on the physiological status of sheep, an alteration common to both treatments is a reduction in the capacity of the animal to respond immunologically to a parasitic invader. It is suggested that reduced immunological responsiveness by the treated sheep may have been the factor which permitted the greater percentage of unarrested development of *Ostertagia* spp. as compared with that in the control animals. It is postulated that in normal sheep which have had no contact with *Ostertagia* spp. or related parasites, a rapid, probably local, immunity reaction leads to the formation and release of factors causing a specific arresting of development of many of the parasites. Although the larvæ were introduced into the rumen by a single injection it is probable that entry to the abomasum will extend over a period of possibly up to 48 hr. It may be that the first larvæ which reach the abomasum cause a local immunity reaction leading to the arrested development of later arrivals.

[1] Dunsmore, J. D., *Nature*, **186**, 986 (1960).

HEART DIPOLE REGIONS AND THE MEASUREMENT OF DIPOLE MOMENT

By Dr. M. R. BARBER
Naval Research Laboratory
AND
Dr. E. J. FISCHMANN
Cardiology Department, Green Lane Hospital, Auckland, New Zealand

Measurement of Specific Dipole Regions within the Heart

MOST electrocardiograph leads used to-day do not differentiate between dipoles from various parts of the heart, but instead give a mixture of all dipoles in unknown proportions. Fischmann and Barber[1] have shown that this applies to so-called 'unipolar' chest leads no less than to standard lead I. If it is assumed that there are N dipole regions in the heart, each of which can be represented by a single dipole, then it is possible to make an electrolytic tank analogue of homogeneous conductivity enclosing a heart model of somewhat higher conductivity. The N dipoles can be placed in the appropriate positions on the heart model. If N electrodes are now distributed over the body surface it is possible to measure N sets of N potentials as each of the dipoles $a,b,c,\ldots$, etc., is excited to unit strength in turn.

Alternatively, if all the N dipoles are excited to different strengths simultaneously and N voltages are measured in the same positions on the body surface, then it is possible to calculate the strengths of each of the N individual dipoles by solving N linear simultaneous equations. The coefficients in the equations are obtained from the $N \times N$ set of potentials described above. The dipole strengths will appear in the form:

$$\mu_a = a_1.V_1 + a_2.V_2 + a_3.V_3 + \ \ldots\ + a_n.V_n$$

where V_1, V_2, V_3, etc., are the body surface potentials.

In practice it is convenient to use the formulæ to design resistor networks which perform automatically the additions of element voltages to give μ_a, μ_b, μ_c, etc. By switching to a particular network it is then possible to display the effects of a chosen dipole on a two-terminal electrocardiograph. The effects of all other dipoles will be eliminated.

Work applying this method to the human torso is in progress and will be published. Accuracy will depend obviously on how closely the analogue represents the electrical properties of the human body under investigation.

Clinical Method for the Quantitative Measurement of the Heart's Total Dipole Moment

THE electrical phenomena associated with the human heart may be attributed to a group of dipoles arbitrarily orientated within a homogeneous medium and producing a set of potentials V_1, V_2, V_3, $\ldots$ V_n at n points on the body surface. An application of Green's reciprocity theorem[2,3] makes it possible to measure the collective dipole strength[4] by applying currents at each of the n points and measuring the fields at the dipole positions.

For example, if the dipoles of strengths μ_1, μ_2, μ_3, $\ldots$ μ_n establish potentials V_1, V_2, V_3, $\ldots$ V_n on the surface, then field strengths E_1, E_2, E_3, $\ldots$ E_n, will exist at the dipole positions when equal currents of magnitude I are applied to each of the n points, where:

$$I(V_1 + V_2 + V_3 + \ldots V_n) = \mu_1E_1 + \mu_2E_2 + \mu_3E_3 + \ldots \mu_nE_n$$

If the field in the region of the dipoles is uniform and Z directed (E_z) then the resultant Z component of all the dipoles may be written:

$$\sum_1^n \mu_z = \frac{I.\Sigma V}{E_z} \qquad (1)$$

Since the dipoles are likely to be orientated randomly it is desirable to establish three mutually perpendicular fields and thereby measure the three resultant dipole components. The main problem is to measure the electric field since the interior of the body is generally inaccessible. This can be resolved, however, by arranging that the flow of current from the electrode is uniform and calculable throughout the body.

If a homogeneous three-dimensional body is box-shaped, it is obviously possible to establish a uniform field within it by applying a uniform current density over one pair of opposite faces. If the conducting medium is replaced by a rectangular three-dimensional fine-mesh resistor network then a similar result will be obtained if equal currents are applied to all leads emerging from one pair of opposite faces. If an irregular-shaped body is now removed from within the box then the uniform field within the body can be preserved if the original currents are maintained at the newly exposed boundaries, that is, equal currents in one direction but zero on the other two.

The situation described can be approximated on the human torso if electrodes are uniformly spaced on a flat platform placed on the chest. Similar electrode clusters can be used for sides and back although for the back electrode it is usually sufficient to use electrodes mounted on a flexible sheet[5]. The electrodes protruding from the platforms can be sprung so that they press firmly on to the body contours.

The approximately uniform field within the body between a pair of such electrodes is simply:

$$E_z = \rho.i$$

where ρ is the conductivity of the body, assumed uniform, and i is the average current per unit area projected on to the platform.

The expression may also be written as:

$$E_z = \frac{\rho.I}{A_{xy}} \qquad (2)$$

where I is the current applied to each of the elements of the cluster and A_{xy} is the small rectangular area enclosing one and extending half-way in all directions to the adjacent element. Combining equations (1) and (2) we obtain:

XUM

$$\sum_{1}^{n} \mu_z = \frac{\Sigma V}{\rho} \cdot A_{xy}$$

as the resultant z component of the dipoles distributed throughout the chest region.

The field produced by the electrode will obviously not be exactly uniform if the electrode elements are spaced at finite intervals and cover a limited area. However, with a frontal cluster having 30 elements and covering an area 10 in. × 14 in. it has been found from model experiments that the fields in the heart region are calculable to within 10 per cent assuming the body to be perfectly homogeneous.

A paper describing, in detail, an orthogonal electrocardiograph lead system, which employs platform-mounted multiple electrodes, will be published[6]. Using a new non-paste electrode material developed by Fischmann *et al.*[7], consisting of balsa-wood impregnated under vacuum with lithium chloride, leads *X*, *Y* and *Z* take as much time to record as three standard electrocardiograph leads.

[1] Fischmann, E. J., and Barber, M. R., *Scientific Exhibit, 129th Annual Conference of Brit. Med. Assoc.*, Auckland, N.Z. (1961).

[2] Green, G., *The Application of Mathematical Analysis to the Theory of Electricity and Magnetism* (Nottingham, 1828).

[3] Smythe, W. R., *Static and Dynamic Electricity*, p. 57 (McGraw-Hill, New York, 1950).

[4] Gabor, D., and Nelson, C. V., *J. App. Phys.*, **25**, 413 (1954).

[5] Reynolds, jun., E. W., Cordes, J. F., Willis, P. W. III, and Johnston, F. D., *Circulation*, **14**, 48 (1956).

[6] Barber, M. R., and Fischmann, E. J., *Brit. Heart J.* (in the press).

[7] Fischmann, E. J., Sealy, R., and Crutcher, R. (unpublished work).

CATION-EXCHANGE CAPACITIES OF TISSUES OF HIGHER AND LOWER PLANTS AND THEIR RELATED URONIC ACID CONTENTS

By A. H. KNIGHT, W. M. CROOKE and R. H. E. INKSON

Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen

SINCE the early work of Devaux[1], which showed that plant tissues possess cation-exchange properties, many workers have measured the cation-exchange capacity (C.E.C.) of the roots of higher plants[2]. Their findings are that dicotyledons have roughly twice the C.E.C. of monocotyledons and that the C.E.C.'s of the common agricultural crops lie in the range 10–60 m.equiv./100 gm. dry matter. Much of the recent interest in this topic stems from the possible effect of C.E.C. on the mineral composition of the plant. In particular, if Donnan distribution principles hold, the higher the root C.E.C. the higher would be the sorption of divalent over monovalent cations (valence effect). The characteristic differences in calcium and potassium contents of monocotyledons and dicotyledons—the former low in calcium and high in potassium, the latter high in calcium and low in potassium—are in keeping with the measured differences in their root C.E.C.

The majority of measurements of C.E.C. of roots have been made on agriculturally important[3] or ornamental plants[4] grown in nutrient culture (nutrient solution, sand or gravel culture) for ease of recovery of roots. These solutions are generally too concentrated to allow the valence effect to be reflected in the plant composition. Under these conditions it has been found that the C.E.C. of a particular species can be altered to some extent by change in nitrogen[5], phosphorus[6] or heavy metal supply[7].

The indiffusible anions in the plant roots to which the C.E.C. could be attributed were demonstrated to be pectic substances by Mattson[8], using staining tests. Decarboxylation methods of estimating uronic acids, supported by chromatographic identification of extracted uronic acids, were used by Keller and Deuel[9] for plant roots and by Knight *et al.*[10] for disks of plant storage tissue. It was shown that a large part of the C.E.C. could be accounted for as pectic substances.

Brief reports on the C.E.C. of other organs (leaves and stems) of higher plants[11], and on the C.E.C.–uronic acid relationship[12] have been published recently. The exchange properties of lower plants have been noted (marine algae[13], yeasts[14], bacteria[15], fungi[16], mosses[17]) but not in relation to uronic acid content.

An adequate test has never been published of the influence of soil factors on the C.E.C. of plants. Earlier work had shown that it was possible to recover plant roots from soil[18], and it was therefore decided to examine the effect of soil-type on the C.E.C. and uronic acid content of natural vegetation. Such plants, since they grow under very poor nutritional conditions, also seemed more likely to offer experimental material which might show the relationship between root C.E.C. and mineral composition.

Plants were therefore collected from sites varying widely in drainage, *p*H and calcium supply. The sites are described more fully in Table 1. In all, about 80 species were collected, but only 12 (5 monocotyledons and 7 dicotyledons) were common to all 4 soil types. Tops were separated into leaves and stems (where appropriate), dried overnight at 80° C. and major element cations determined in the ash. After washing, root systems were divided into tap roots, rhizomes and fine roots (where appropriate), dried overnight at 80° C. and milled to pass a 0·7 mm. sieve. The C.E.C. was measured using an acid-washing technique followed by titration to *p*H 7 using 0·01 *N* potassium hydroxide[7]. Uronic acids were estimated by a micro-decarboxylation method[19], and the results are expressed in m.equiv./100 gm. assuming all carbon dioxide was derived from carboxyl groups.

Fig. 1 shows the relationship found between the uronic acid content and the C.E.C. of the roots. The diagonal line represents the theoretical 1 : 1 rela-

Table 1. Description of Sites

Soil type	Site	*p*H	% Loss on ignition	Exchangeable cations m.equiv./100 gm. oven-dry soil Ca	K
Sand	Newburgh, Aberdeenshire	4·8–6·8	—*	—*	—*
Peat	Gardrum Moss, near Falkirk	4·0	72	12·9	0·75
Acid soil	Craigiebuckler, Aberdeenshire	6·5	6	7·5	0·39
Calc. soil	Limehillock, Banffshire	8·0	12	55·0†	0·38

Additional sites: sand, Maryculter and Glendye, Aberdeenshire; peat, Mossmaud and Lyne of Skene, Aberdeenshire; acid soil, Bridge of Alford, Aberdeenshire; calcareous soil, Blackhillock, Banffshire.
* Not determined; † This soil yielded 15·3 per cent CO_2, equivalent to 34·8 per cent $CaCO_3$.

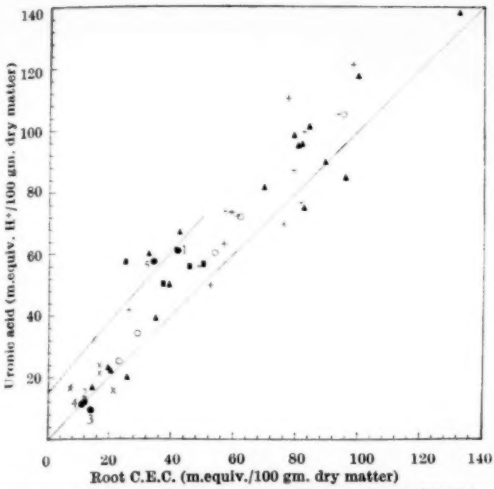


Fig. 1. Relationship between cation-exchange capacity (C.E.C.) and uronic acid content (in m.equiv./100 gm.) for the roots of higher plants. Lower plants are indicated thus: ferns (4, ■), mosses (15, +), marine algae (18, ▲), and fungi (6, ×). Numbered points are 1, liverwort; 2, lichen; 3, bacteria; 4, yeast; 5, *Equisetum*. Open circles are Keller and Deuel's results.

tionship. The short solid line is derived by the method of least squares from the results of 62 samples, embracing the 12 angiosperm species ranging in C.E.C. from 6 to 50 m.equiv./100 gm. dry roots, which were common to all 4 soil types. The total of 62 samples is explained by the fact that there were several sampling sites for each type of soil. Table 2 gives the mean values for these 12 plants. Statistical examination shows that there are no significant differences between the individual regression lines calculated from the data for each of the 4 soil types.

The slope and intercept of the line in Fig. 1 show that C.E.C. is, in general, accounted for by uronic acid content, but there is included in the ordinate a proportion of esterified carboxyl groups which yield carbon dioxide, but do not take part in cation exchange. The effect of interfering substances on the decarboxylation results has been discussed[10,19].

It was found that the C.E.C.–uronic acid relation also held for different parts of the same plant. For example, for *Heracleum* sp. the values (in m.equiv./100 gm. dry matter) for C.E.C. and uronic acid are, respectively: roots, 25·8, 49·5; tap root, 26·0, 52·0; stem, 26·7, 52·5; leaves, 26·3, 70·7. It would appear that leaves contain a higher proportion of substances which yield carbon dioxide but do not take part in cation exchange.

To extend the data to include gymnosperms, the roots of 23 species of one-year-old conifer seedlings growing on the acid soil were also examined and the C.E.C. (17·9–30·8, mean 25·3) and uronic acid values were found to fall on the same line as the Angiosperms, but for clarity these results are not plotted. For comparison with the results of other workers, values obtained by Keller and Deuel[9], using somewhat different techniques of plant culture and analysis, have been plotted. These are shown by open circles and are for the roots of 4 crop plants and one ornamental plant, larkspur[20], which gives the highest value.

Having established the line for the higher plants, it was decided to perform tests on a few species from the other major groups in the plant kingdom. These results have been plotted on Fig. 1 and are made up of: ferns (4, ■), mosses (15, +), marine algae (18, ▲), fungi (6, ×). Single samples of liverwort, lichen, soil bacteria, yeast, and *Equisetum* sp. are indicated by solid circles numbered 1–5, respectively. These determinations were carried out on whole plants except in the case of ferns and *Equisetum* where roots were used. All samples were oven-dried, then milled as for the higher plant roots.

It would appear that the C.E.C. and its related uronic acid value is a characteristic of the roots of angiosperms and is independent of a wide range of growth conditions. The C.E.C.–uronic acid relationship is also shown by other organs of the plant. For lower plants there also seems to be a general relationship between C.E.C. and the decarboxylation value of the dry matter. Different substances may be involved in the production of carbon dioxide for the different plant groups, and 'uronic acid' is used only as a convenient overall expression. It is reasonable to suppose that the uronic acid in the roots of angiosperms is mainly polymerized galacturonic acid (in other words, pectic substances) but little is known of the occurrence of these compounds in the lower plants. In brown algae, the uronic acid is probably alginic (that is, a polymer of mannuronic acid). From the results obtained here, it is noteworthy that among the marine algae the C.E.C. values fell into two clear groups, those of the brown algae being 70 and above and those of the red and green algae 56 m.equiv./100 gm. dry matter and below. The presence of ethereal sulphate in seaweeds which would contribute to C.E.C. but not to the decarboxylation value would obviously affect the relationship.

Table 2. Average Values (in m.equiv./100 gm.) of C.E.C. and Uronic Acid (U.A.) Content of Plant Roots Grown in Different Soil Types

Growth medium	Sand		Peat		Acid soil		Calcareous soil	
	C.E.C.	U.A.	C.E.C.	U.A.	C.E.C.	U.A.	C.E.C.	U.A.
Monocots. (5 spp.)	13·2	28·1	12·3	27·2	12·6	24·8	13·3	28·5
Dicots. (7 spp.)	25·0	45·7	32·8	49·1	27·2	48·3	30·8	48·0

We thank R. Boggie, E. Birse, Miss M. E. K. Henderson and Dr. H. G. M. Hardie for their assistance, J. E. Shackleton, of Ben Reid's Nursery, Aberdeen, for supplying the conifer seedlings, and E. Booth, of the Institute of Seaweed Research, Inveresk, for samples of marine algae.

1 Devaux, H., *C.R. Acad. Sci., Paris*, **133**, 58 (1901).
2 Drake, M., and Campbell, J. D., *Proc. Amer. Soc. Hort. Sci.*, **67**, 563 (1956); Williams, D. E., and Coleman, N. T., *Plant and Soil*, **2**, 243 (1950); Graham, E. R., and Baker, W. L., *Soil Sci.*, **72**, 435 (1951); Smith, R. L., and Wallace, A., *Soil Sci.*, **81**, 97 (1956); Blanc-Aicard, D., *Ann. Agron.*, **4**, 615 (1955); Helmy, A. K., and Elgabaly, M. M., *Plant and Soil*, **10**, 78 (1958).
3 Drake, M., Vengris, J., and Colby, W. G., *Soil Sci.*, **72**, 139 (1951).
4 Dunham, C. W., Hamner, C. L., and Asen, S., *Proc. Amer. Soc. Hort. Sci.*, **68**, 556 (1956).
5 McLean, E. O., *Soil Sci. Soc. Amer. Proc.*, **21**, 219 (1957).
6 Huffaker, R. C., and Wallace, A., *Agron. J.*, **51**, 118 (1959).
7 Crooke, W. M., *Soil Sci.*, **86**, 231 (1958).
8 Mattson, S., Eriksson, E., Vahtras, K., and Williams, E. G., *LantbrHögsk. Ann.*, **16**, 457 (1949).
9 Keller, P., and Deuel, H., *Z. PflErnähr. Düng.*, **79**, 119 (1957).
10 Knight, A. H., Crooke, W. M., MacDonald, I. R., and Shepherd, H., *J. Exp. Bot.*, **12**, 13 (1961).
11 Inden, T., Hori, S., and Okuda, A., *Nippon Dojo Hiryogaku Zasshi*, **29**, 380 (1958), (*C.A.*, **54**, 21579b).
12 Cecconi, S., Toninelli, F., and Radaelli, L., *La ricerca scientifica*, **28**, 605 (1958).
13 Eppley, R. W., *Exp. Cell. Res.*, **13**, 173 (1957).
14 Rothstein, A., and Hayes, A. D., *Arch. Biochem. Biophys.*, **63**, 77 (1956).
15 McCalla, T. M., *J. Bact.*, **40**, 23 (1940).
16 Susman, A. S., and Lowry, R. J., *J. Bact.*, **70**, 675 (1955).
17 Puustjarvi, V., *Arch. Soc. Vanamo*, **9**, 257 (1955).
18 Crooke, W. M., and Knight, A. H. (unpublished results).
19 Tracey, M. V., *Biochem. J.*, **43**, 185 (1948).
20 Keller, P., Diss., Tech. High. Sch. (Zurich, 1960).

IMPORTANCE OF OSMOTIC POTENTIAL AS A COMPONENT OF THE TOTAL POTENTIAL OF THE SOIL WATER ON THE MOVEMENT OF NEMATODES

By C. D. BLAKE

Rothamsted Experimental Station, Harpenden, Hertfordshire

THE force with which water is held in a soil can be specified in terms of a total water potential (ψ), identified as the free energy of the soil water, and is conveniently expressed as $pF = \log_{10}(-\psi)$. This total potential is the sum of a matric potential or suction (h), caused by hydrostatic pressure deficiencies existing in the soil water, and an osmotic potential (π), caused by dissolved salts.

Horizontal migration of the nematodes, *Heterodera schachtii* Schmidt and *Ditylenchus dipsaci* (Kühn) Filipjev, through sands wetted with distilled water is affected by the matric potential applied to the sands[1]. In these conditions the total potential equals the matric potential, that is, the suction applied, and there is no information on the effects of osmotic potential. I therefore compared the movement of *D. dipsaci* larvæ in a sand of particle size 250–500μ with and without an osmotic potential. In one lot of experiments the sand was saturated with distilled water and suctions of 0–100 cm. water applied. The larvæ that moved horizontally more than 3 cm. in 5 hr. through the sand column were counted and expressed as a percentage of the number of larvæ introduced. In the other experiment the sand was saturated with a 0·1 *M* solution of urea and suctions of 0–100 cm. water applied.

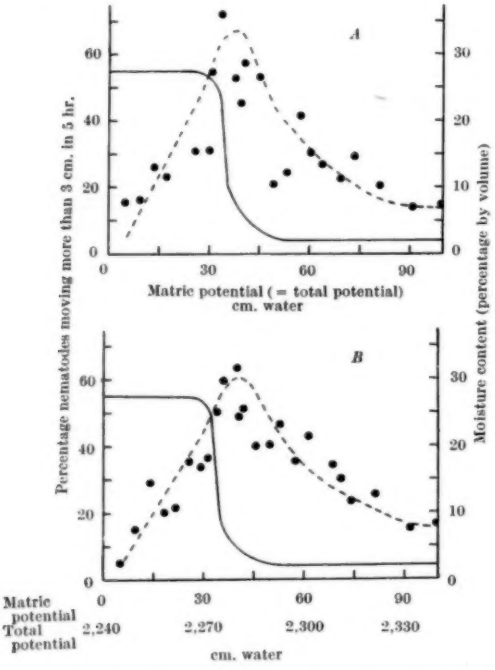


Fig. 1. Horizontal migration of *D. dipsaci* through sand at different total potentials. Each point is the mean of two replicates. In *A* the sand is wetted by distilled water (osmotic potential = 0) and in *B* by 0·1 *M* urea solution (osmotic potential = 2,240 cm. water). The moisture characteristic of the sand is shown as a solid curve

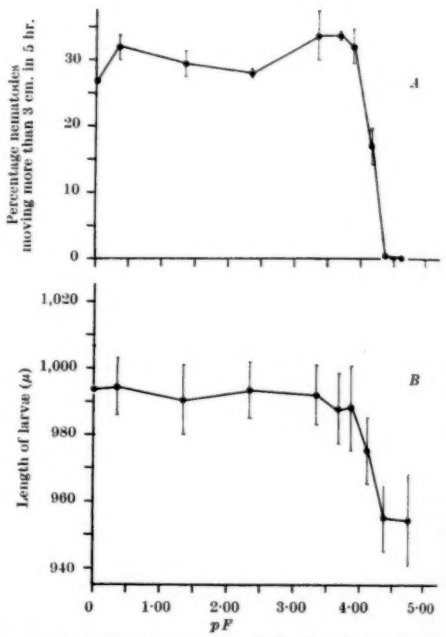


Fig. 2. *A*, Horizontal migration of *D. dipsaci* through sand wetted with urea solutions of different concentrations. Each point is the mean of five replicates and the standard error of each mean is shown by a solid line through the point. *B*, Length of *D. dipsaci* larvæ measured after immersion for 36 hr. in urea solutions of different concentrations. Each point is the mean of fifty measurements and the standard error of each mean is shown by a solid line through the point

Fig. 1 shows that the larvæ moved similarly whether the sand was wetted with distilled water or urea solution; movement in both was related to the moisture characteristic and was fastest as the pores of the sand began to drain, showing that movement depends only on the moisture content and is independent of the osmotic potential. The extrusion of the mass of larvæ of *Anguina agrostis* (Steinbuch) Filipjev from flower galls was also sensitive to suction but unaffected by changes in osmotic potential within the range of 0–300 cm. water[2].

The mobility of *D. dipsaci* larvæ was also measured in 250–500μ sand saturated with solutions of 0·0001 *M*–2 *M* urea and drained by a suction of 32 cm. water. The percentage of larvæ that moved more than 3 cm. in 5 hr. was not influenced by the osmotic potential of the sand solution until the concentration exceeded

Table 1. Expected Changes in the Total Potential of a Typical Soil. Moisture Content 20 per cent w/w, after Addition of Dextrose at Six Levels/Acre to a Depth of 8 in.

Rate of application of dextrose to soil		Matric potential of soil (cm. water) (h)	Osmotic potential of soil before dextrose added (cm. water) (π_1)	Total potential of soil before dextrose added (cm. water) ($\psi_1 = h + \pi_1$)	Molar concentration of dextrose in soil water	Osmotic potential of dissolved dextrose (cm. water) (π_2)	Total potential after dextrose added ($\psi_2 = \psi_1 + \pi_2$)	
Per cent w/w	Tons/acre						cm. water	$pF = \log_{10}(-\psi_2)$
0·1 (1,000 p.p.m.)	1	75	400	475	0·028	627·2	1102·2	3·04
1	10	75	400	475	0·28	6272	6747	3·83
2	20	75	400	475	0·56	12544	13019	4·11
3	30	75	400	475	0·84	18816	19291	4·29
4	40	75	400	475	1·12	25088	25563	4·41
5	50	75	400	475	1·40	31360	31835	4·50

0·3 M ($pF = 3·87$), when it decreased and movement ceased at 1 M ($pF = 4·35$) (Fig. 2*A*).

The length of the fourth-stage larvæ of *D. dipsaci* suspended in urea solutions decreased as the osmotic potential of the solutions increased.

Shrinkage was greatest in the range of 0·001–1 M concentration and the larvæ reached their minimum lengths at pF approximately 4·1 (Fig. 2*B*). Larvæ in the 1 M and 2 M urea solutions were killed, but those transferred to distilled water after 36 hr. in solutions of 0·001 M–0·3 M urea regained their activity and former length.

Dextrose and three salt solutions ranging from 0·2 to 1 M depressed hatching of eggs of *H. rostochiensis* Woll. and *Meloidogyne arenaria* (Treub) Chitwood, but in all experiments hatching was resumed when the eggs were transferred to water[3]. Emergence of larvæ from cysts of *H. schachtii* similarly decreased rapidly as the solute concentration exceeded 0·01 M regardless of the solute used[4]. The speed of movement of *D. dipsaci* declined when the solute concentration exceeded 0·1 M and the movement of *D. dipsaci* and the emergence of *H. schachtii* both ceased at about 1 M ($pF = 4·35$). This potential approximates to the permanent wilting point of plants.

Matric potential affects nematode migration by varying the thickness of the water films surrounding the soil particles[5]. Figs. 1 and 2 show that the rate of movement of *D. dipsaci* in sand depends only on the matric potential and is independent of the osmotic potential until the solute concentration is such as to cause incipient plasmolysis of the nematode. In most fertile soils the salt concentration of the soil water does not exceed 0·05 M ($pF = 3·05$) and so nematode movement through a soil is normally governed not by the total potential but by the matric potential of the soil system.

Sucrose or dextrose applied to soil at the rate of 1–5 per cent by weight of soil has been claimed to be nematicidal and proposed as a method for controlling plant parasitic nematodes in field-crops[6]. Assuming the dextrose was thoroughly mixed in a soil of moisture content 20 per cent w/w to a depth of 8 in., that the osmotic potential of the soil water before treatment was equivalent to 400 cm. water and that the matric potential of the soil was 75 cm. water, it is of interest to calculate the changes in pF of the soil after adding dextrose at the rates of 1–5 per cent by weight suggested (Table 1).

The growth of most plants becomes slower as the total potential exceeds $pF = 3·30$ and stops at $pF = 4·00$ (ref. 7). With 0·1 per cent dextrose, the resultant pF of 3·83 may retard plant growth, but from Fig. 1 such a total potential would probably not slow nematode movement. The pF at which nematodes are killed by ex-osmosis approximates to the pF at which plants permanently wilt. Unless sugar were applied only during a period of bare fallow, it is difficult to see practical uses for the treatment if it were economic. Even during bare fallow, such large quantities of pure carbohydrate could be expected to render nitrogen temporarily unavailable and induce profound changes in the soil microbial community.

This work was done while I held the Sir Benjamin Fuller Trust scholarship and was on leave from the Department of Agriculture, Sydney, Australia.

1 Wallace, H. R., *Ann. App. Biol.*, **46**, 86 (1958).
2 Collis-George, N., and Blake, C. D., *Austral. J. Biol. Sci.*, **12**, 247 (1959).
3 Dropkin, V. H., Martin, G. C., and Johnson, R. W., *Nematologica*, **3**, 115 (1958).
4 Wallace, H. R., *Ann. App. Biol.*, **44**, 274 (1956).
5 Wallace, H. R., *Ann. App. Biol.*, **46**, 74 (1958).
6 Feder, W. A., *Pl. Dis. Reptr.*, **44**, 883 (1960).
7 Russell, E. J., *Soil Conditions and Plant Growth*, eighth edit. (Longmans, Green and Co., London).

CHROMOSOMAL ABERRATIONS INDUCED BY AN ANIMAL VIRUS

By Dr. BERGE HAMPAR and Dr. SOLON A. ELLISON
Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York

Several agents known to cause mutation of mammalian cells have also been shown to induce chromosomal aberrations[1–4], and it is generally accepted that such aberrations represent one stage of the mutagenic process induced by these agents. No similar effect on chromosomes has been shown in the case of animal viruses, although there is evidence implicating viruses as possible mutagens which can produce heritable changes in one or more cell characteristics[5–8]. This article describes the effect of herpes simplex virus on the cytology of chromosomes in a line of Chinese hamster (*Cricetulus griseus*) cells in tissue culture. In this instance, infection produced cytological changes similar to those previously described as the result of treating mammalian cells with X-irradiation[1], alkylating agents[2,3], and bromodeoxyuridine[4].

The cell line used (designated *MCH*) appeared spontaneously in a bottle seeded with a normal 23-chromosome line of Chinese hamster cells received from Dr. G. Yerganian, and designated by him as 44–*FAF*–10[9]. No cellular proliferation had been evident for 8 days after seeding. Since healthy-appearing cells were still present, the culture was trypsinized and passaged to several T-flasks. Slow proliferation of the passaged cells was noticed during

Fig. 1. Chromosomes of an uninfected *MCH* cell. M_1 and M_2 indicate marker chromosomes

several days, and these cells have now been maintained for 30 passages. Their generation time is at present similar to that of normal Chinese hamster cells (approximately 14 hr.). The *MCH* line is characterized as hyperdiploid, containing 23–28 chromosomes, and approximately 5 per cent of the cells are in the polyploid region (29 or more chromosomes). In comparison with chromosomes of normal Chinese hamster cells which have been examined in considerable detail[10–12], the unique characteristic of the *MCH* line is the presence of a marker chromosome not previously seen (Yerganian, G., personal communication). Fig. 1 shows the chromosomes of an uninfected *MCH* cell, in this instance containing two marker chromosomes differing slightly in their length. The sex chromosomes in the illustration are essentially normal, but slight variations in the X_1 and X_2 chromosomes have been observed. The marker chromosome is found in all cells regardless of chromosome number.

Cells were grown in a mixture consisting of Puck's medium (36 per cent), Hanks' balanced salt solution (37 per cent), N.C.T.C.–109 (6 per cent), lactalbumin hydrolysate (0·5 per cent), horse serum (10 per cent) and fœtal calf serum (10 per cent). The medium also contained penicillin (50 units/ml.) and streptomycin (50 μgm./ml.). All experiments were run in a humidified atmosphere containing 10 per cent carbon dioxide. For karyotype analysis, $2{\cdot}5 \times 10^5$ cells were inoculated into 50 mm. Petri dishes containing 2 coverslips. Colchicine (1 μgm./ml.) was added after appropriate intervals and the cultures were incubated for an additional period, generally 6 hr. Carnoy's solution and acetocarmine were used for fixing and staining. Squash preparations were made according to the method described by Yerganian (in the press). In those experiments involving viral infection, the inoculum was added to the cells when the cultures were initiated. Mitotic indices and plating efficiency studies were carried out in parallel with chromosome analysis.

The strain of herpes simplex virus was isolated in 1952 on the chicken chorioallantoic membrane[13] and had been stored at − 70° C. until 1960, when it was adapted to growth in HeLa cells. The 49th and 50th tissue culture passages were used.

We have confirmed Yerganian and Gresser's finding (Yerganian, G., and Gresser, L., personal communication) that normal Chinese hamster cells are not susceptible to infection by herpes simplex virus. When the *MCH* line is inoculated with this agent, no cytopathic effect is observed nor do characteristic intranuclear inclusions develop, but chromosomal aberrations are seen during the first cell division following infection. Virus can be recovered from the supernatant culture fluids during two cell passages following infection—even though the cells are repeatedly washed free of released virus—as well as from frozen-thawed cells after three subsequent passages. Viral infection at the time of plating does not alter the cloning efficiency, which is approximately 80 per cent. Clones formed in the presence

Fig. 2. Chromosomes of an infected *MCH* cell. *A*, chromatid break on chromosome 1 which has translocated to a medium-sized metacentric; *B*, chromatid exchange involving chromosome 2 and the X_1 chromosome; *C*, centromeric fusion of two telocentric chromosomes; *D*, chromatid separation and translocation involving the X_2 chromosome

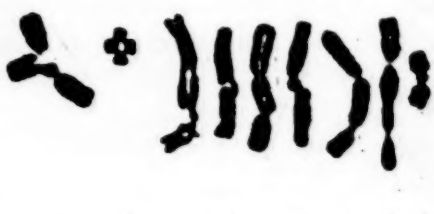


Fig. 3. Examples of chromosomal aberrations in infected *MCH* cells: *a*, chromatid break at the centromere of chromosome 2 showing terminal association with the short arm of the X_1 chromosome; *b*, reciprocal (chromatid) translocation involving two small metacentric chromosomes at their centromeric regions; *c*, chromatid deletion of chromosome 1 with segmental interchange; *d*, chromatid break at centromeric region of chromosome 1; *e*, *f*, chromatid break along chromosome 1; *g*, *h*, chromosome dicentric or exaggeration of secondary constrictions on chromosome 1; *i*, centromeric fusion of two telocentric chromosomes

of virus consist of moderately ballooned cells in contrast to the characteristically long and narrow fibroblastic cells seen in uninfected clones. These results warrant the assumption that a type of carrier culture has been produced.

Figs. 2 and 3 illustrate the types of chromosomal changes produced by virus infection. (We are indebted to Dr. G. Yerganian for his assistance in interpreting the karyotypes.) Most aberrations occur at the centromeric and secondary constriction regions of chromosomes 1 and 2 as shown in Figs. 2 and 3*a*, *c*, *d*, *e* and *f*, as well as at the centromeric regions of subtelocentric chromosomes, with the resultant formation of acentric fragments. Where definitive breaks do not occur, exaggeration or elongation of centromeric and secondary constriction areas are evident (Figs. 3*g* and *h*). Fusion at the centromeric regions of two chromosomes (Fig. 3*i*) is seen infrequently.

Table 1 presents a compilation of the chromosomal aberrations that result when cultures are infected at a multiplicity of one. Chromatid breaks are common in infected cultures whereas uninfected controls and cultures inoculated with heat-inactivated virus show only chromosome breaks. Translocations are recorded as to the number of cells, and these cells are not included in other categories. Aberrations are not restricted to any particular karyotype, and are observed in cells bearing varied chromosome numbers. Heat-inactivated virus shows no effect, as indicated in Table 1. Aberrations seem to be restricted to the metaphase of the first cell division following infection, even though virus is present during several subsequent passages. The mitotic indices of infected and uninfected cultures show no significant variation, both reaching a maximum in approximately 40 hr. after initiation of the cultures.

Table 1. CHROMOSOMAL ABERRATIONS IN NORMAL AND VIRUS-INFECTED *MCH* CELLS

Age (hr.) when colchicine added*	No. of cells scored	Chromatid and chromosome breaks per cell	Accentuated secondary constriction or centromeric regions per cell	Percentage of cells with translocations
Infected				
2	102	0·18	0·07	3·9
14	200	0·23	0·08	9·5
40	196	0·21	0·12	4·1
72	218	0·09	0·007	0·0
Uninfected or inoculated with heat-inactivated virus				
2–72†	400	0·075	0·002	0·75

* Colchicine treatment lasted 6 hr. except in the case of 2-hr.-old cultures which were exposed to colchicine for 18 hr.
† No variation with time observed in these cultures.

The chromosomes of cells no longer releasing virus are now being examined in detail. Preliminary work indicates that if chromosome variations are present after infection, they are not qualitative but rather quantitative or cryptostructural[14]. The only variations noted thus far between infected and uninfected cells are based on slight morphological alterations which cannot in themselves be considered conclusive proof of induced mutation. The fact, however, that virus infection does not result in any recognizable cell destruction and that a short-term carrier state can be established in the *MCH* line, indicates that the aberrations seen are non-lethal. It is therefore possible that some permanent alteration in cellular characteristics has occurred.

This work was supported by a research contract [*Nonr* 266(63)] between the Office of Naval Research and Columbia University, and by a grant from the Bristol-Myers Company. One of us (B. H.) is a post-doctoral Research Fellow of the National Institute of Dental Research, National Institutes of Health.

[1] Puck, T. T., *Proc. U.S. Nat. Acad. Sci.*, **44**, 772 (1958).
[2] Yerganian, G., and Livingston, C. A., *Proc. Amer. Assoc. Cancer Res.*, **2**, 159 (1956).
[3] Yerganian, G., *Proc. Intern. Genetics Symp.*, 1956, 206 (*Cytologia* Supp., 1957.
[4] Hsu, T. C., and Somers, C. E., *Proc. U.S. Nat. Acad. Sci.*, **47**, 396 (1961).
[5] Vogt, M., and Dulbecco, R., *Proc. U.S. Nat. Acad. Sci.*, **46**, 365 (1960).
[6] Medina, D., and Sachs, L., *Virology*, **10**, 387 (1960).
[7] Dulbecco, R., and Vogt, M., *Proc. U.S. Nat. Acad. Sci.*, **46**, 1617 (1960).
[8] Stoker, M., and MacPherson, I., *Virology*, **14**, 359 (1961).
[9] Yerganian, G., and Leonard, M. J., *Science*, **133**, 1600 (1961).
[10] Ford, D. K., and Yerganian, G., *J. Nat. Cancer Inst.*, **21**, 393 (1958).
[11] Ford, D. K., Wakonig, R., and Yerganian, G., *J. Nat. Cancer Inst.*, **22**, 765 (1959).
[12] Yerganian, G., *Cytologia*, **24**, 66 (1959).
[13] Ellison, S. A., Thesis, Columbia Univ. (1958).
[14] Levan, A., *Ann. N.Y. Acad. Sci.*, **63**, 774 (1956).

INFLUENCE OF THE AGE OF POLLEN AND STIGMAS ON SEX DETERMINATION IN HEMP

By REGINA LASKOWSKA
Institute of Soil Science and Plant Cultivation, Pulawy, Poland

PREVIOUS investigations on the influence of the age of pollen on sex determination in hemp have given conflicting results[1,2]. The possibility of regulating the number of the male and female individuals in a population would have great practical significance. For this reason investigations were carried out during 1955–60 concerning the influence of the time of storing of pollen as well as the age of stigmas on sex determination in hemp derived from Poland, a type intermediate between that of central Europe and central U.S.S.R.

Female plants of hemp, with different or similar ages of stigmas, were pollinated with pollen of determined age in the same doses. Plants which were pollinated naturally were used as control series. In the next generation the relations of sex were analysed in all the combinations. In 1955 the age of stigmas was not taken into consideration. The female flowers in full blossom, that is, with stigmas of different ages, were pollinated with pollen in three stages of ripeness: (1) gathered from flowers immediately after dehiscence; (2) stored in uncovered Petri plates in free air for 6 hr.; (3) stored in the same way for 12 hr. In the experiment in 1956 both the ages of pollen and that of stigmas were known. Female plants with stigmas 7–12 days old were pollinated with pollen: (1) fresh, gathered immediately after opening of male flowers; (2) stored in uncovered Petri dishes in the laboratory for 24 hr. The experiments were carried out in a greenhouse with artificial or space isolation. The results are summarized in Table 1.

The plants begin to blossom gradually on different nodes; for this reason the age of stigmas may be very different in the female flowers flowering at the same

Table 1

Stigmas	Pollen	Year	Number of plants in 1st generation			Per cent plants in 1st generation		Number of plants in 2nd generation			Per cent plants in 2nd generation	
			Total	*M*	*F*	*M*	*F*	Total	*M*	*F*	*M*	*F*
All ages	fresh	1955/56	535	224	311	41·9	58·1	—	—	—	—	—
7–12 days old	,,	1956/57	736	256	480	34·8	65·2	—	—	—	—	—
1–3 days old	,,	1958/59	242	90	152	37·3	62·7	284	130	159	45·8	54·2
12 days old	,,	1958/59	36	14	22	38·9	61·1	47	12	35	25·5	74·5
All ages	kept for 6 hr.	1955/56	663	262	401	39·5	60·5	—	—	—	—	—
,,	kept for 12 hr.	1955/56	648	290	358	44·7	55·3	—	—	—	—	—
7–12 days old	kept for 24 hr.	1956/57	696	307	389	44·1	55·9	—	—	—	—	—
1–3 days old	kept for 14–17 days	1958/59	158	45	113	28·4	71·6	212	93	119	43·9	56·1
12 days old	kept for 14–17 days	1958/59	22	7	15	31·8	68·2	—	—	—	—	—
control		1955/56	550	242	258	44·0	56·0	—	—	—	—	—
,,		1956/57	700	315	385	45·0	55·0	—	—	—	—	—
,,		1958/59	138	67	71	48·5	51·5	166	83	83	50·0	50·0

time. It was observed that the hemps investigated began to bloom at the 7th or 8th leaf node (counting the cotyledon node as the first). On the basis of this observation, in the experiments of 1958 only the flowers at the 7th and 8th nodes were pollinated, that is, those which had stigmas 1–3 and 12 days old. Pollination was carried out with fresh, and with 14–17-days-old, pollen. In this experiment the mother plants were sown on the ground and covered with double sacks of dense fabric, while pollen was kept in a desiccator with calcium chloride. Plants which were pollinated naturally were used as controls, but seeds were taken from the 7th and 8th node only. Both in controls and in most of the combinations with fresh pollen the seeds were set normally; only in combinations with stigmas 12 days old and fresh pollen, and stigmas 12 days old with pollen 14–17 days old (Table 1), was the number of seeds considerably less, and consequently the number of plants in these series was relatively small.

Nevertheless the results of these two series were also taken into account when the conclusions were drawn, since similar sex-ratios were observed in previous years.

From Table 1 one may conclude that: (1) the age of the stigma does not influence the direction of sex-determination in hemp; (2) depending on the period of storage, pollen has a certain influence on the numerical relations of male and female individuals of the given population.

In the first and second generations a predominance of female plants occurred when pollination was carried out with fresh pollen (immediately after the beginning of pollen emission by the flowers and up to 6 hr. afterwards) and when pollen was 14–17 days old. In crosses which were pollinated with fresh pollen the number of female plants was about 2–11 per cent higher in the first generation and 13 per cent higher in the second than in the control series. When pollination was performed with pollen 14–17 days old the majority of female plants in relation to the control series amounted to 20 per cent in the first generation and 5 per cent in the second. Pollen 12 and 24 hr. old did not cause any changes in the sex ratio, the results being identical with those obtained in the control series.

The results of investigations suggest that: (1) The rhythm of development of pollen with female tendency (x) and of that with male tendency (y) is different. (2) It is possible that in pollen populations with male tendency a certain percentage of grains ripens later than those of the populations with female tendency In consequence, a shift of direction of sex ratio towards the female side may occur in the populations of plants derived from pollination with young pollen. (3) The preponderance of female plants after the pollination with pollen 14–17 days old may be evidence either of the frequent occurrence of longer duration of vitality of pollen with female tendency, or (and it means the same in the case of phenotypic observations) of shorter duration of vitality of some part of pollen with male tendency. (4) The ratio of male plants to female plants near to 1 : 1 obtained in the populations derived from pollination with pollen 12 and 24 hr. old may be due to equal quantities of pollen of both tendencies at this age and, in this case, to the random process of pollination.

[1] Bessey, E. A., *Amer. J. Bot.*, **15**, 405 (1928).
[2] Ciesielski, T., *Quomodo fiat, ut mox proles masculina, mox feminina oriatur apud plantas, animalia et homines?* (Leopolis, Lwów, 1911).

SIMULATED WAVE-RIDING DOLPHINS

By BYRNE PERRY
Department of Civil Engineering, Stanford University, Stanford, California
AND
A. J. ACOSTA and TARAS KICENIUK
Hydrodynamics Laboratory, Division of Engineering, California Institute of Technology, Pasadena

THE explanation of the mechanism by which dolphins are able to ride bow waves of ships, or natural surf and wind-generated waves, has stimulated much discussion and controversy. Since an excellent summary of previous research on the wave-riding problem has been given by Fejer and Backus[1], only a few remarks by way of review will be given here.

Although dolphins had been observed riding on bow waves of ships many times over the years, Woodcock[2,3] published the first account which included considerations of the forces estimated to be required. Shortly thereafter, Woodcock and McBride[4] undertook to explain in more detail the nature of these forces. They found that a 200-lb. dolphin submerged in sea water would have, at most, a net

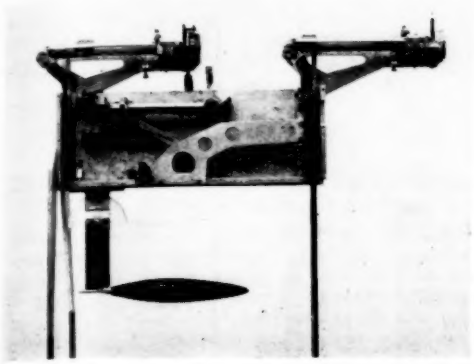


Fig. 1. The 'dolphin-like body' mounted on the 3-component force balance used in these experiments

weight of 9·2 lb. The component of this weight in the direction of the local flow was thus shown to be too small to overcome the expected viscous drag, unless the dolphin was in some manner able to maintain a laminar flow over its body-surface. The Reynolds number for these observations was $8{\cdot}4 \times 10^6$ based on body-length. For rigid bodies tested under many conditions it is known that at such a Reynolds number the boundary layer is almost certainly turbulent; hence, the conclusion would follow that the dolphin had achieved the surprising feat of maintaining a laminar boundary layer (and hence, very low drag) at a very high Reynolds number.

It was Hayes[5] who pointed out that it was the component of the total weight rather than the net weight in the direction of the local flow that provided the thrust. With this force available, there is no need to suppose the dolphin has a viscous drag appreciably lower than any other streamlined body moving at the same Reynolds number.

In spite of the lucid arguments set forth by Hayes, some workers remain unconvinced. Scholander's experiments in the open sea[6] convinced him that the Hayes explanation was not satisfactory. Scholander and Hayes[7,8] thereupon arrived at an impasse, with Scholander suggesting that further experiments be conducted under better-controlled conditions.

In discussing these considerations with Prof. Scholander, it occurred to us that the free-surface water tunnel in the Hydrodynamics Laboratory at this Institute could be used to help resolve this controversy.

Apparatus and Experimental Procedure

At tunnel operating speeds approximating 7 ft./sec., it is possible to establish a stable, standing wave in the working section of the free-surface water tunnel[9]. Such a wave was formed, and the simulated dolphin model (consisting of a streamlined body of revolution 2 in. in diameter and approximately 1 ft. long) was immersed in the wave and the hydrodynamic reactions were measured. The test body, strut support, and force balance assembly are shown in Fig. 1. Extensive force measurements had already been made on this body in previous investigations[10] using wind tunnel, water tunnel and drop tank facilities, and were therefore available for comparison. The configuration of this body, however, had been slightly modified for the present experiments by replacing the original tail and boom assembly with a shorter one. Also, an adjustable streamlined strut had been attached to this boom, thereby permitting the attitude of the test body to be determined within an angle of $\frac{1}{2}$ degree.

Prior to starting the tunnel, tare forces were measured with the test body in air, at each of the angles of the test body used in the experiment. By means of an elevating mechanism, the balance and body assembly were lowered until the centre-line of the body was at a depth of about 0·2 ft. The buoyant force acting on the body in still water was then measured. The tunnel was started and the standing wave established. Force readings at the two tunnel locations were made for angles of 0° and $\pm 8°$ at various submergences.

Position 1 (Fig. 2) was located on the upstream slope of the wave, considerably ahead of the crest. It had been hoped that the body could be located somewhat farther downstream; but this could not be done easily because of interference with a tunnel structural member. Position 2 (Fig. 3) was in the trough of the wave. The water velocity in these tests was of necessity near the critical channel velocity, but varied locally with position along the wave. A miniature *HRS* current meter about $\frac{1}{2}$ in. in diameter was used for measuring these velocities at the same longitudinal position and depth as the midpoint of the test body. These results are summarized in Table 1 as well as are some other conditions not described.

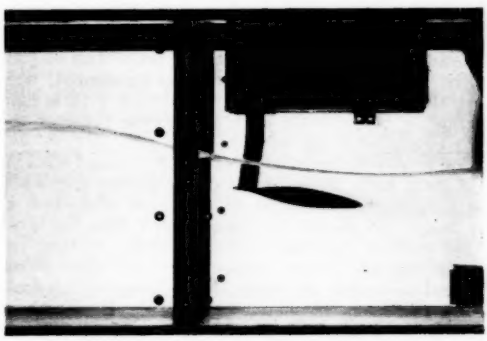


Fig. 2. 'Dolphin-body' located at tunnel position No. 1 on the forward slope of a standing wave. Net forward thrust = 0·007 lb. at a velocity of 7·02 ft./sec.

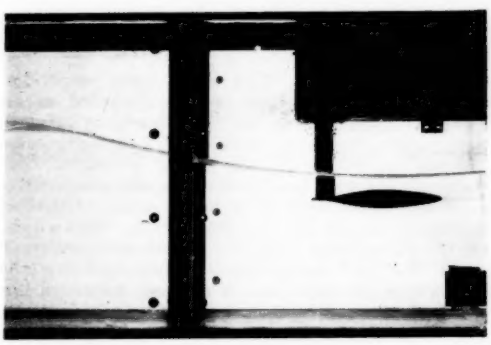


Fig. 3. 'Dolphin-body' located at tunnel position No. 2 in the trough of the standing wave. Net drag = 0·071 lb. at 7·24 ft./sec. Note the dye streak ahead of the model near the model centre-line

Table 1

Run No.	Tunnel position	Angle	Depth	Drag*	Lift	Water velocity	Remarks
1	1	−10 deg.	0·25 ft.	0·000 lb.	0·774 lb.	0·00 ft./sec.	
2	1	0	0·1	0·000	0·776	0·00	
3	1	0	0·2	0·000	0·777	0·00	Stationary
4	1	− 8	0·25	0·000	0·776	0·00	
5	1	+ 8	0·15	0·000	0·778	0·00	
6	1	0	0·2	0·031	0·889	2·78	Flat free surface
7	1	0	0·2	0·032	0·775	3·54	(no standing wave)
8	2	0	0·2	0·071	0·842	7·24	
9	2	+ 8	0·25	0·106	0·900	7·22	In trough of wave
10	2	− 8	0·05	0·070	0·829	7·26	
11	1	+ 8	0·2	0·059	1·022	7·08	
12	1	0	0·2	0·001	0·870	7·12	
13	1	− 8	0·1	0·008	0·641	7·02	
14	1	− 8	0·15	−0·011	0·748	7·08	Forward slope of wave
15	1	− 8	0·2	−0·007	0·793	7·02	
16	1	− 8	0·25	−0·009	0·800	7·04	
17	1	− 8	0·35	−0·001	0·821	7·02	
18	1	− 8	0·45	0·006	0·824	7·00	

* Negative values imply thrust.

Observations and Analysis of Results

When the test body was aligned with the slope of the water surface and submerged beneath the upstream slope of the wave in position 1, it was immediately apparent that a small, forward thrust between 0·007 and 0·011 lb. was measured (see runs 14–17). However, when the body was located in the trough of the wave at a similar submergence (runs 8, 9, 10) the body experienced a drag force ranging from 0·07 to 0·106 lb. These figures can be compared with the still-water buoyant force of 0·78 lb. to establish scale. Although no attempt was made to find the optimum location of the body with respect to the wave in so far as thrust is concerned, it is clear that a net thrust can be achieved with a rigid streamline body under even the gentle slope of the wave produced in the tunnel.

The observed thrust varies with depth and changes to a drag both when the body broaches and when the submergence exceeds about three diameters of the body. It should be remembered that the drag of the strut is included in these results so that the present results do not reflect the body drag alone. The submergence for least drag observed herein—about one body diameter—is roughly the same as that reported for dolphins in bow waves.

Further insight into the mechanics of the force system acting on the body is obtained by resolving the forces along axes parallel to and perpendicular to the local flow angle[5]. For example, for run 14 of Table 1 the resistance parallel to the local flow is 0·054 lb. and the force normal to the flow (or free surface) is 0·745 lb., with the estimated local flow angle of 5°. This latter force, perpendicular to the wave surface, can be regarded as an inclined buoyant force as mentioned by Hayes. These forces, it must be recalled, are hydrodynamic in origin and do not include the body-weight. Let it be supposed now that the self-propelling capability of a body, the weight of which is equal to that of the displaced fluid, is to be determined. This weight of 0·777 lb. resolved along the direction of flow must exceed the flow resistance of 0·054 lb. For the present example, this force is not only exceeded but there is also, in fact, a net propelling force of 0·013 lb. available. The forces are not precisely in equilibrium for this example; the body, if free, would tend to sink to a slightly greater depth, where the vertical force is seen to be larger.

The flow resistance of 0·054 lb. resolved along the local flow-angle is roughly comparable with the drag measured in the trough of the wave (run 8) and both are typical of viscous drag measurements in a turbulent flow[9,11]. Laminar skin friction is therefore seen not to be a necessary condition for bow wave-riding as was originally contained in the suggestion by Woodcock.

It is also interesting to observe that the vertical forces, both in the slope of the wave and in the trough, differ noticeably from the buoyant force in still water. This is thought to be due to a combination of two effects: the well-known repulsion experienced by a submerged body moving under an otherwise flat, free surface, and more important, the curvature of the local streamlines. Summary calculations have shown the contributions of the latter term to the lift force to be of the correct magnitude.

Conclusion

The mere existence of the measured forward-acting thrust on the test body in a wave demonstrates that a rigid body with turbulent flow can perform the wave-riding trick. Analysis of the measurements shows further that the force system is equivalent to a dynamic buoyant force perpendicular to the inclined wave surface and a resistance parallel to this surface. The dynamic buoyancy is approximately equal to the weight of the displaced liquid, while the viscous resistance is substantially equal to that measured on the body when not in the wave.

In spite of these incomplete experimental results, it appears inescapable that Hayes's arguments are substantially correct.

This work was supported, in part, by the Department of the Navy, under Contract No. 123 (60530) 24917*A*.

[1] Fejer, A. A., and Backus, R. H., *Nature*, **188**, 700 (1960).
[2] Woodcock, A. H., *Science*, **133**, 952 (1961).
[3] Woodcock, A. H., *Nature*, **161**, 602 (1948).
[4] Woodcock, A. H., and McBride, A. T., *J. Exp. Biol.*, **28**, 215 (1951).
[5] Hayes, W. D., *Nature*, **172**, 1060 (1953).
[6] Scholander, P. F., *Science*, **129**, 1085 (1959).
[7] Scholander, P. F., *Science*, **130**, 1658 (1959).
[8] Hayes, W. D., *Science*, **130**, 1657 (1959).
[9] O'Neill, J. P., *The Hydrodynamics of the Free-Surface Water Tunnel*, Rep. No. *N*-65, Hydrodynamics Laboratory, California Institute of Technology, Pasadena (1949).
[10] Kermeen, R. W., and Wald, R. L., *Water Tunnel and Drop Tank Tests of a Streamlined Body of Revolution*, Rep. No. *E*-61, Hydrodynamics Laboratory, California Institute of Technology, Pasadena (1954).

LETTERS TO THE EDITORS

SPACE SCIENCE

Errors in Orbital Predictions for Artificial Satellites of Earth

SEVERAL workers[1-3] have carefully investigated variations in the accelerations of artificial satellites of Earth, but no one seems to have published a systematic study of the errors in orbital predictions. The results of such work[4] are presented in this communication. A stochastic model was constructed which takes into account fluctuations in atmospheric density, observational errors, and the correlations introduced by smoothing. Errors in actual predictions issued by the Vanguard Computing Center, National Aeronautics and Space Administration Computing Center, Smithsonian Astrophysical Observatory, and Naval Weapons Laboratory were also computed, for comparison with the theoretical model.

The actual and theoretical errors in orbital predictions are given in Tables 1 and 2 and Fig. 1. Table 1 contains the errors in 1–2-week predictions made near the peak of the sunspot cycle. Fig. 1 presents the same data graphically. Table 2 shows the errors in predictions half-way between sunspot maximum and sunspot minimum. In Tables 1 and 2, N is the number of revolutions predicted, beginning at the centre of the smoothing interval. The smoothed rate of change of period is dP/dn (min./revolution). The root-mean-square prediction error, E (min.), includes the contributions of observational errors and drag fluctuations. The theoretical prediction error caused by observational errors alone is designated by E_0.

It is interesting to note that observational errors were the principal cause of errors in orbital predictions for only one of the cases shown in Fig. 1, that of *Vanguard I* with its perigee in darkness (winter 1959–1960). In all the cases shown in Fig. 1, the prediction errors attributable to observational errors were smaller than the total error for *Vanguard I* in darkness. If the errors in the predictions had been caused only by observational errors, then the prediction errors would have been independent of the smoothed rate of change of period. A detailed discussion of the theory and the method of calculation is given in ref. 4.

Table 1. ERRORS IN PREDICTION NEAR PEAK OF SUNSPOT CYCLE

Satellite	Dates	No. of predictions	$-dP/dn$ (min./rev.)	N (rev.)	E_0 (min.)	E Actual (min.)	E Theoretical (min.)
Explorer IV	1958	8	$2{\cdot}15 \times 10^{-3}$	165	0·024	3·2	3·3
Sputnik III	1958	7	$1{\cdot}32 \times 10^{-3}$	220	0·01	3·3	1·9
Vanguard I	Autumn 1958	20	$5{\cdot}5 \times 10^{-5}$	154	0·056	0·25	0·22
Vanguard I	Summer 1959	11	$2{\cdot}1 \times 10^{-4}$	154	0·056	0·13	0·097
Vanguard I	Winter 1959–1960	7	$6{\cdot}5 \times 10^{-4}$	154	0·056	0·062	0·061
Atlas-Score	Dec. 1958–Jan. 1959	1*	$2{\cdot}2 \times 10^{-2}$	271	0·3	67	74

* A single observation has no statistical significance. This case is included merely to show how large the error can be when the rate of change of period is large.

Table 2. ERRORS IN PREDICTION HALF-WAY BETWEEN SUNSPOT MAXIMUM AND MINIMUM

Satellite	Dates	No. of predictions	$-dP/dn$ (min./rev.)	N (rev.)	E_0 (min.)	E Actual (min.)	E Theoretical (min.)
Tiros II	Dec. 1960–May 1961	12	$3{\cdot}7 \times 10^{-4}$	250	0·08	0·12	0·08
Vanguard I	Oct. 1960–May 1961	12	$7{\cdot}4 \times 10^{-4}$	150	0·06	0·12	0·06
Transit III-B	Feb.–Mar. 1961	10	$1{\cdot}05 \times 10^{-3}$	22	0·04	0·74	0·50
Echo I	Oct–Dec. 1960	0	$6{\cdot}8 \times 10^{-4}$	145	0·04	4·4	3·3

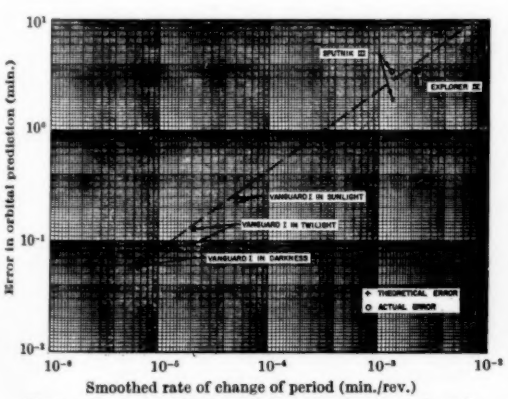


Fig. 1. Errors in orbital predictions running for 1–2 weeks

KENNETH MOE

Space Technology Laboratories, Inc.,
P.O. Box 95001,
Los Angeles 45, California.

[1] Jacchia, L. G., *Smithsonian Astrophysical Observatory Special Report* No. 39 (1960).
[2] Priester, W., and Martin, H. A., *Mitteilung der Universitäts-Sternwarte Bonn*, No. 29 (1960).
[3] Paetzold, H. K., *A Proposal for a Self-Consistent Model of the Upper Atmosphere* (Technische Hochschule, München, 1961).
[4] Moe, K., *TR-60-0000-09145* (Space Technology Laboratories, Inc., April 1960).

ASTROPHYSICS

Secular Variations of Short-lived Sunspots

LARGE secular variations have been found in short-lived sunspots as recorded in "Sunspots seen on one Day Only" of the Greenwich Photo-Heliographic Results, 1879–1957. The 11-yr. period often appears like a subsidiary perturbation.

A pertinent example is seen in Fig. 1, which shows the ratio K between the number of one-day spots at less than 65° from the central meridian, and the number of spots seen throughout two days or more. In Fig. 2 is plotted the number of one-day spots without umbra as a percentage of the total number.

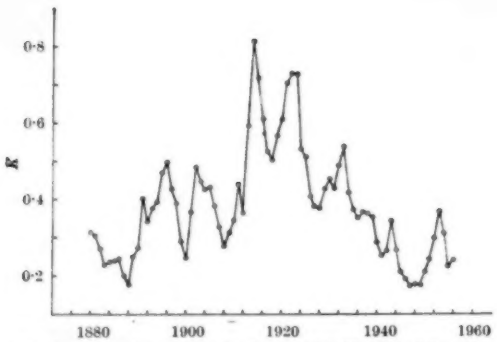


Fig. 1. Sliding 3-yr. mean for the period 1880–1956 of the ratio between the number of one-day spots and the number of spots observed throughout two or more days

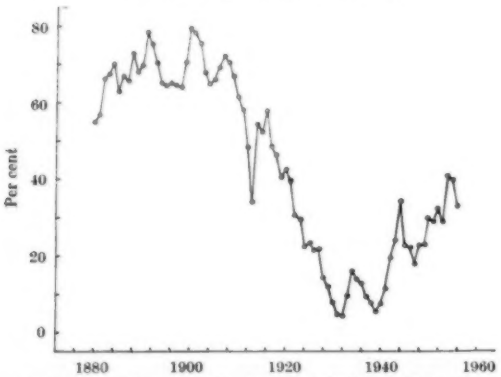


Fig. 2. Sliding 3-yr. mean for the period 1880–1956 of the number of one-day spots without umbra as a percentage of total spot number

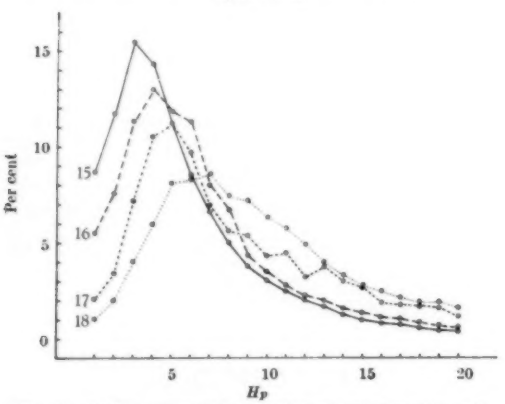


Fig. 3. Sliding mean of the number of one-day spots with determined total area as a percentage of total numbers for cycle 15 (1914–24), cycle 16 (1922–34), cycle 17 (1932–45) and cycle 18 (1942–54)

The frequency distribution with respect to area (both total area and area of the umbra) varies from one cycle to the next, and in such a way that the frequency maximum decreases with increasing area. This is shown in Fig. 3, which gives the smoothed frequency distribution with respect to total area for the solar cycles 15–18.

In an earlier paper[1] secular variations were found in the relation between magnetic field-strength H and total area A_p of sunspots, $H = a.\log A_p + b$, where a and b are coefficients that vary systematically from one cycle to the next. A connexion was predicted between the secular variations and variations in number and distribution of area of short-lived spots. This is borne out by the present investigation, which shows a marked correlation between the coefficients a and b and the mean values of K and A_p for one-day spots in the various cycles.

The research is supported in part by the Geophysics Research Directorate, Office of Aerospace Research, United States Air Force.

T. S. Ringnes
Institute of Theoretical Astrophysics,
University of Oslo,
Blindern-Oslo.

[1] Ringnes, T. S., and Jensen, E., *Astrophysica Norvegica*, 7, 99 (1960).

RADIOPHYSICS

Coincidence of the Explosive Phase of Solar Flares with 10·7-cm. Solar Noise Bursts

The observational evidence of an explosive phase in the development of the solar flare, as reported by Athay and Moreton[1], is of great interest. This phase appears after the commencement of the flare, is well defined, and thus provides a stage in the evolution of certain flares which may be of significance for examination of flare-related phenomena, such as solar noise bursts, etc. The Athay and Moreton paper lists eleven selected flares with 'explosive' phases. Two of these flares occurred outside the normal observing period at Ottawa, but the remaining nine can be compared with 10·7-cm. solar noise bursts observed at Ottawa. These cases, while few in number, sharpen the already well-established close relationship between flares and 10·7-cm. radio bursts which have been derived from other work done on the Ottawa data[2].

The relationships between the starting times of the flare, the explosive phase (as given in Table 1, of ref. 1) and the starting time of the 10·7-cm. burst (as given in the "Tabulation of the 10·7-cm. Outstanding Occurrences" published in the *CRPL–F*, Part *B, Solar-Geophysical Data*) are shown in Fig. 1. In order to provide identification, each radio event has been assigned the same letter as the associated flare event. The upper histogram shows the distribution of the difference in time of start of the flare and of the explosive phase; the lower histogram shows the dis-

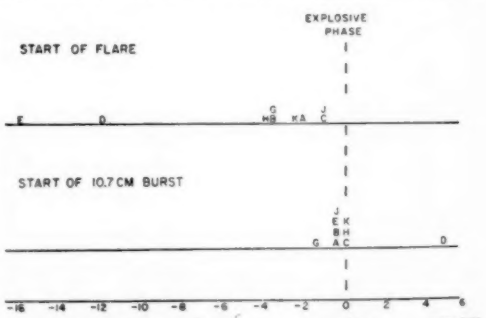


Fig. 1. Upper, distribution of the difference in time of start of the flare and of the explosive phase; lower, distribution of the difference in time of start of the 10·7-cm. burst and the explosive phase of the flare

tribution of the difference in time of start of the 10·7-cm. burst and of the explosive phase of the flare. Comparison of the two histograms shows that, on the average, the start of the 10·7-cm. burst precedes the explosive phase of the flare by approximately 1/2 min. (excluding event *D*) whereas the start of the burst follows the commencement of the flare by approximately 2 min. (excluding events *E* and *D*).

The coincidence of the time of start of the burst and the time of the explosive phase of the flare is not altered significantly when the criterion used to determine the start of the burst is examined. Burst commencement is that time when the rising flux has departed from the steady background-level by an amount equal to the residual receiver noise. In each of the nine cases examined here, the bursts are of the impulsive type, with a well-defined and rapid increase of emission from the steady background to a peak. Variations in interpretation and timing errors could introduce an uncertainty in the time of the start of the bursts of no more than ± 1/2 min.

Examination of the time profile of the 10·7-cm. burst shows that there are two basic types of bursts: the impulsive burst, and the long-enduring burst (gradual rise and fall)[2]. As mentioned above, the bursts which are here associated with the explosive phase of the flare are all of the impulsive type. They are all, also, of more than average intensity. It may be of some significance that, in every case but one, the impulsive bursts are superimposed on long-enduring enhancements of radio emission of lesser intensity, and that in the one exception the occurrence of sunset precludes any observation.

The occurrence of an impulsive burst on a long-enduring enhancement is regarded as a single composite event from the frequency with which the association appears. In the large number of cases which have been observed, the separation of the commencements of the impulsive burst and the long-enduring enhancement range from a few minutes to several hours. In the extreme, when the two bursts presumably start at the same time, two types of profiles have been observed in the resultant composite event, and have been classified differently in the published 10·7-cm. burst data. When the maximum of the long-enduring burst appears after the occurrence of the impulsive burst, the composite event is classified as a long-enduring burst with the impulsive burst superimposed on it. However, when the impulsive burst is followed by a long-enduring enhancement which shows only a steady decline, the event is classified as a single impulsive burst with a post-burst increase.

In the case of the nine radio events associated with the flares with explosive phases, both of these types of composite events have appeared. In cases *D* and *K*, the impulsive bursts are superimposed on long-enduring bursts which commence 23 min. before the impulsive event, and continue for approximately 1 hr. after the end of the impulsive burst. Burst *D* is the only impulsive feature in this composite event, but burst *K* is the third and most intense, by a factor of 3, of a group of three impulsive features in this composite event. Radio event *H* is a period of irregular activity in which a great number of impulsive events of various intensities appear superimposed on an elevated background. The most intense of these impulsive events has an intensity which is 17 times greater than the next largest of the superimposed features, and it is this intense impulsive feature which

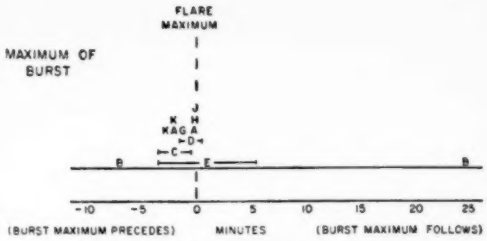


Fig. 2. Distribution of the difference in time of 10·7-cm. burst maximum and the time of flare maximum

corresponds in time with the explosive stage of the flare. The period of irregular activity starts 6 min. before and ends approximately 30 min. after this impulsive burst. In case *J*, sunset coincides with the final decline of the impulsive burst, and, while no long-enduring enhancement of emission was in evidence just prior to the burst, it is impossible to know whether any enhancement followed it. In all the other cases, *A*, *B*, *C*, *E*, and *G*, the impulsive bursts are followed by relatively long-enduring enhancements of emission of much less intensity (post-burst increase).

The comparison of the time of burst maximum with the time of flare maximum is shown in Fig. 2. In the case of complex bursts, the times of both maxima are indicated by the letter identifying the event. In the cases where the burst produced off-scale readings and the maxima were not recorded, the off-scale period is indicated by a line. Although this histogram shows more scatter than that of the start of the bursts in relation to the explosive phase, it indicates that burst maximum is reached shortly before flare maximum in general.

The preceding detailed analysis involving a group of flares selected for the explosive phase in their evolution shows that the time of start of the 10-cm. burst coincides with the explosive phase rather than the commencement of the flare, and these bursts, without exception, are of the impulsive type. A second type of 10-cm. burst, the long-enduring burst (gradual rise and fall), cannot be directly related to this feature. This suggests that it is related to some other feature in the evolution of the flares.

Arthur E. Covington
Gladys A. Harvey

Radio and Electrical Engineering Division,
National Research Council, Ottawa.

[1] Athay, R. G., and Moreton, G. E., *Astrophys. J.*, **133**, 3 (1961).
[2] Dodson, H. W., Hedeman, E. R., and Covington, A. E., *Astrophys. J.*, **119**, 3 (1954).
[3] Covington, A. E., and Harvey, G. A., *J. Roy. Astro. Soc., Canada*, **52**, 161 (1958).

OCEANOGRAPHY

Sea Surface Temperature in Coastal Waters of the British Isles

Mr. A. J. Lee has recently commented[1] on my paper dealing with variations of sea surface temperature in coastal waters of the British Isles. He points out that I made no mention of the part played by currents in determining the winter pattern of sea surface temperature. I was, however, primarily concerned with the *shape* of the isotherm patterns. The presence of the relatively shallow water of the Dogger Bank area will, in itself, ensure that the pattern will consist of two tongues, although the level

of temperature of these tongues will be largely determined by the strength of the currents mentioned by Mr. Lee.

Mr. Lee also criticizes the statement that the English Channel is isothermal or very nearly so throughout the year. Taking this statement in isolation, the criticism is fully justified; but it was intended to be taken as a comment on Fig. 1(*b*), which shows the mean temperature profiles at a position (49° 49′ N., 02° 33′ W.) towards the middle of the English Channel. Perhaps this should have been made clearer in the text.

I did not discuss the accuracy of the observations because the paper was written as an aid to local weather forecasting, and for this purpose an accuracy of ± 0·5° C. is adequate. Most of the sea temperatures used were obtained by the bucket method, and it is generally recognized that the above-mentioned standard of accuracy can be obtained by this method, if the readings are taken with reasonable care. Measurements of sea temperature made recently on Ocean Weather Ship *Monitor* by a thermistor, supplied by the Fisheries Laboratory, Lowestoft, and floated astern of the ship, steaming slowly at about 2 knots or when stopped 'on station', at a distance of 10 m., show that the differences from the bucket measurements rarely exceed 0·5° C. (only 1 occasion in 34 over a period of four days). There is always a danger of much larger errors of reading, for example, 5°, but as shown in my paper these can be readily distinguished if the sea temperature isotherms are drawn intelligently.

F. E. Lumb

Meteorological Office,
London Road,
Bracknell, Berkshire.

[1] *Nature*, **190**, 1164 (1961).

PHYSICS

X-Ray and Neutron Diffraction Examination of *p*-Diphenylbenzene

The crystal structure of *p*-diphenylbenzene was first determined by Pickett[1], using projection X-ray diffraction techniques. Because of the considerable theoretical interest in the bond-lengths of conjugated carbon-carbon bonds[2,3] a refinement of the structure has been commenced, using full three-dimensional X-ray and two-dimensional neutron diffraction techniques.

The refinement of the data on *h*0*l* neutron diffraction projection has given some interesting results, which are discussed here. The neutron intensity data were collected on the single crystal spectrometer installed at the *Hifar* reactor at the Australian Atomic Energy Commission Establishment. The intensity of the neutron beam from the (1,1,1) plane of a copper monochromating crystal was 140,000 counts/sec./cm.^{2}, when set to reflect neutrons with a wave-length of 1·0015 ± 0·0005 Å. The intensities at all the *h*0*l* reflexions out to sin θ/λ = 0·8 Å.$^{-1}$ were measured, and of the 248 possible reflexions, 139 had intensities sufficiently greater than the incoherent scattering background. By comparison, in the *h*0*l* projection in the X-ray case, 107 of the 140 possible reflexions out to the copper $K\alpha$ limit were obtained.

The *h*0*l* projections of both the neutron and the X-ray structure have been refined simultaneously. This has enabled a comparison to be made of the relative power of the two methods. The X-ray structure determinations showed that the thermal motions of a number of the atoms in the molecule were markedly anisotropic. Significant peaks were found in the difference synthesis at positions corresponding to the hydrogen atoms, when these were not included in the structure factor calculation. From this synthesis approximate positions of the hydrogen atoms could be determined. The resolution obtained was poor, however, there being considerable overlapping of the features due to thermal anisotropy with those caused by hydrogen atom contribution. Consequently, an accurate evaluation of the thermal parameters and the positions of the hydrogen atoms was not possible. The corresponding neutron diffraction projection showed much better resolution and confirmed the X-ray observations. The atomic peaks in the Fourier synthesis[4] (Fig. 1), based on the neutron diffraction data, are well resolved. The form of the peaks gives clear indications of the extent of the anisotropy in the thermal motion of the atoms. The negative peaks corresponding to the hydrogen atoms are well resolved. This has led to an accurate determination of their positional parameters.

Certain interesting features of the structure are obvious from an examination of the neutron diffraction Fourier synthesis. First, there are significant deviations of some of the atoms from their normal positions so that the carbon skeleton of the molecule is slightly distorted, presumably for packing considerations. Each of the benzene rings has its normal configuration, but the two end rings are out of parallel with the central ring by approximately 1° 30′ (Fig. 2). Secondly, several of the hydrogen atoms are distorted from their normal positions due to steric hindrance within the molecule. Thus the hydrogen atoms attached to the C_1 and C_3 atoms (Fig. 2) are displaced in directions which increase the distance between them. This is in accordance with expectations, for if they occupied their normal positions they would be only 1·8 Å. apart, whereas the

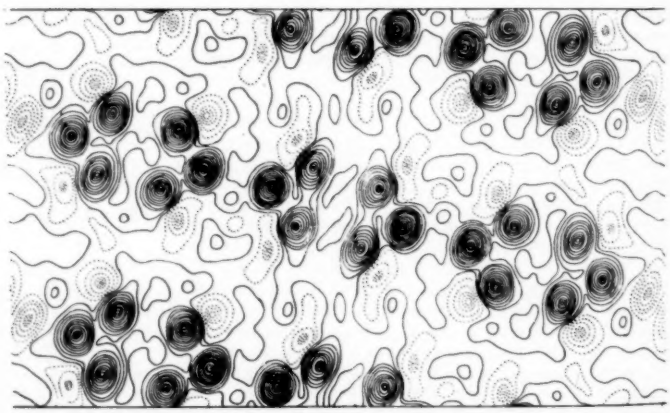


Fig. 1. A Fourier projection on the (010) plane of *p*-diphenylbenzene at room temperature based on neutron diffraction data. Contour intervals are in arbitrary units. Full lines are positive, broken lines are negative contours

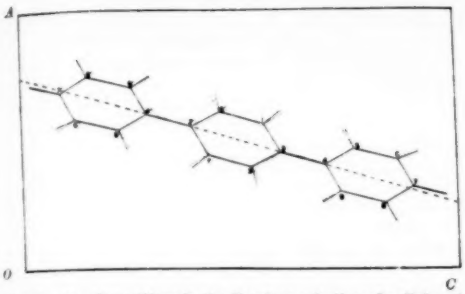


Fig. 2. Atomic positions in the Fourier projection of *p*-diphenylbenzene. Full lines are the undistorted C—H bonds

sum of their Van der Waals radii is 2·4 Å. The equivalent pair of hydrogens attached to the C_2 and C_5 atoms is similarly displaced.

It is also possible from work on the thermal parameters to deduce in projection the predominant modes of thermal vibration within the molecule. The C_2 and C_4 atoms have thermal motions considerably less in magnitude than those of the remaining carbon atoms. The motions of all the atoms are markedly anisotropic, the direction of maximum vibration being perpendicular to the long axis of the molecule. It may be shown from this that two modes of vibration predominate. The first appears in projection as an oscillation about the centre of gravity of the molecule, and the second is an oscillation about the long axis of the molecule, that is, the line joining the C_7 and C_7' carbon atoms. The presence of the former mode confirms the recent suggestions of Bacon and Curry[5] that the outer benzene rings in molecules of this type have considerably more freedom of movement than the inner ones.

The X-ray and neutron diffraction work is continuing, and a full report will be published elsewhere.

The first three authors are indebted to the Australian Institute of Nuclear Science and Engineering for the generous financial assistance that has made the neutron work possible. The co-operation and assistance of the *Hifar* reactor staff is gratefully acknowledged.

C. J. B. Clews
E. N. Maslen
H. M. Rietveld

Physics Department,
University of Western Australia,
Nedlands.

T. M. Sabine

Australian Atomic Energy Commission,
Lucas Heights,
New South Wales.

[1] Pickett, L., *Proc. Roy. Soc.*, **142**, 333 (1933).
[2] Dewar, M., and Schmeising, H., *Tetrahedron*, **5**, 166 (1959).
[3] Mulliken, R., *Tetrahedron*, **6**, 68 (1959).
[4] Bacon, G., and Curry, N., *Acta Cryst.*, **13**, 717 (1960).
[5] Bacon, G., *Neutron Diffraction* (Clarendon Press, 1955).

Energy-Level Model for High-Resistivity Gallium Arsenide

Although gallium arsenide is usually prepared with low resistivities (< 1 ohm-cm.), it has also been prepared for several years with very high resistivities (> 10^6 ohm-cm.). This latter material can be produced by zone refining[1], by the addition of impurities such as oxygen[1], or under growth conditions where the main shallow donor, silicon, is reduced below roughly one part per million[1]. To explain the occurrence of high-resistivity gallium arsenide, Welker[2] suggested that the donors and acceptors are built into the lattice in pairs. More recently, Allen[3] has termed this material 'semi-insulating', and claims that the existence of *n*-type high-resistivity material requires that there be a very high degree of compensation between the shallow donor and a deep acceptor. He also states that because of the frequency of occurrence of high resistivities, some mechanism of automatic compensation must be operative. Later, to explain gradual transitions in resistivity along floating-zone refined ingots, Gooch *et al.*[4] have shown that a wide range of resistivities can still occur even on assuming a high degree of automatic compensation. However, according to their model, the degree of automatic compensation must vary along the ingot, yet cannot vary too much otherwise *p*-type material is predicted, which is not observed.

A three-energy-level model is proposed here which explains the existence of high-resistivity material without requiring that there be a high degree of compensation, and therefore no need for invoking the occurrence of automatic compensation. Similar to the above model[4], a wide range of resistivities will be shown to be possible. The energy-level scheme is shown in Fig. 1, where N_D is the density of shallow donors, N_{DD} the density of deep donors, and N_A is the density of acceptors. It is easily seen that high-resistivity material will result when $N_A > N_D$ and $N_{DD} > (N_A - N_D)$. These conditions are not very restrictive, and explain why high-resistivity gallium arsenide can occur so frequently. For example, for a given concentration of N_{DD}, high resistivity will result when N_D is removed at a faster rate than N_A, as possible in zone refining; or else that the concentration of N_A is increased, which might occur when oxygen is added. Of course, when N_D is somewhat greater than N_A, the material is low-resistivity *n*-type, and the N_{DD}-levels are neutral. It is also seen that so long as $N_{DD} > N_A$, *p*-type material will not occur.

The model of Fig. 1 can be treated simply mathematically assuming that the activation energy of the deep donor is E_d, and that of N_D and N_A is zero; however, relaxing these latter restrictions does not detract qualitatively from the results. Then the electron concentration, n, is given by:

$$n = \frac{N_c N_{DD} \exp(-E_d/kT)}{2n + N_c \exp(-E_d/kT)} + \frac{n_i^2}{n} - (N_A - N_D) \quad (1)$$

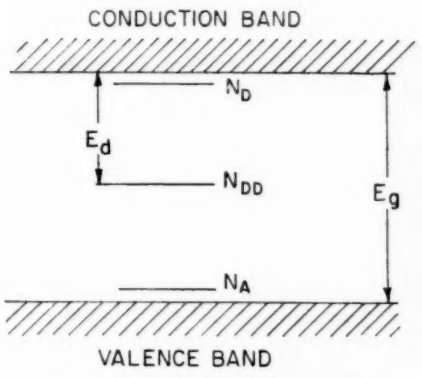


Fig. 1. Energy-level diagram for the model

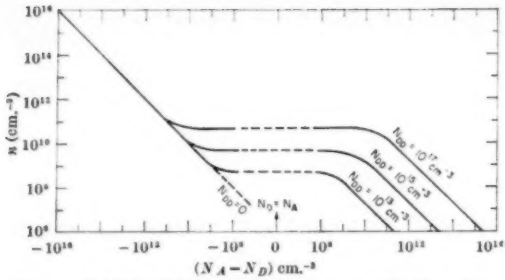


Fig. 2. Variation of electron concentration, n, with $(N_A - N_D)$ at room temperature for various values of N_{DD}, with $E_d = 0{\cdot}76$ eV.

where n_i is the intrinsic electron concentration, and N_c is the effective density of states in the conduction band.

A value for E_d of 0·76 eV. has been deduced by Gooch *et al.*[4]. Using this value, the quantity $N_c \exp(-E_d/kT)$ in the denominator of equation (1) can be neglected for n-type samples and then equation (1) can be easily solved for n. The variation at room temperature of n with $(N_A - N_D)$ is shown in Fig. 2 for $N_{DD} = 10^{13}$, 10^{15}, and 10^{17} cm.$^{-3}$, using $N_c = 6{\cdot}3 \times 10^{17}$ cm.$^{-3}$ and $n_i^2 = 1{\cdot}1 \times 10^{13}$ cm.$^{-3}$. The results of this model are quite similar to those of Gooch *et al.*[4] in that a wide range of resistivities can occur, and further three conductivity-ranges are predicted. At high electron concentrations, the Hall coefficient should be independent of temperature; at very low electron concentrations, the activation energy deduced from Hall effect measurements should give E_d; however, only in the case of extremely close compensation where $N_D \approx N_A$ should the activation energy be $E_d/2$, since in this range the deep donor is effectively uncompensated.

A donor located at the centre of the energy band gap of gallium arsenide has been detected by thermally stimulated current measurement[1,5], and has been found, where experimentally feasible, in every crystal to date at densities of 10^{16} cm.$^{-3}$ or greater. From Fig. 2, it can be seen that the electron concentrations will be between 10^6 and 10^8 cm.$^{-3}$ for values of $(N_A - N_D)$ in the range from 2×10^{15} to 2×10^{13} cm.$^{-3}$, which can easily occur as the shallow donor concentration is reduced. This would also explain why there is almost no p-type gallium arsenide produced in undoped growth of gallium arsenide, but instead why usually either very high or very low resistivity n-type material is found. Actually, thermally stimulated current measurements[1,5] have also shown the presence of other deep donors with shallower levels than 0·76 eV. In this case, the three-level model is an oversimplification, and one would expect that the variation of Hall coefficient and conductivity with temperature would exhibit intermediate energy-levels, especially below room temperature, and this has already been observed[5].

Conductivity versus temperature slopes have been measured in n-type gallium arsenide with activation energies greater than half the band gap, that is, as large as 0·8 and 0·9 eV. (C. Hilsum, private communication; R. H. Bube, private communication). This behaviour is explicable on the present model. In equation (1), it can be shown that at room temperature and above, for $N_{DD} > 10^{11}$ cm.$^{-3}$ and for $E_d > 0{\cdot}8$ eV., both n_i^2 and the term $N_c \exp(-E_d/kT)$ can be neglected. Then so long as $(N_A - N_D)^2 \gg 2 N_C N_{DD} \exp(-E_d/kT)$, n will vary as:

$$n = \frac{N_C N_{DD}}{2(N_A - N_D)} \exp(-E_d/kT) \qquad (2)$$

The above condition is quite easily met. Typical values could be $N_{DD} = 5 \times 10^{16}$ cm.$^{-3}$, $N_D = 10^{12}$ cm.$^{-3}$, and $N_A = 10^{13}$ cm.$^{-3}$, and the sample will still be n-type. It is also interesting that, depending on the magnitudes of $(N_A - N_D)$ and N_{DD}, a sample of gallium arsenide might never become intrinsic, and can exhibit a conductivity or Hall constant versus temperature slope greater than the intrinsic slope up to the melting point.

Finally, it should be noted that the distinction drawn by Gooch *et al.*[4] between 'semi-insulating' gallium arsenide, and 'high-resistivity semiconducting' gallium arsenide prepared by diffusion of copper into low-resistivity material[6], is non-existent in principle on the present model, since the concept of automatic compensation has not been invoked.

We are indebted to Dr. R. H. Bube for helpful discussions, and to Drs. Gooch, Hilsum and Holeman for placing their paper at our disposal prior to publication. This work was sponsored by the Electronics Research Directorate of the U.S. Air Force Office of Aerospace Research under contract.

J. Blanc
L. R. Weisberg

Radio Corporation of America Laboratories,
Princeton, New Jersey.

[1] Weisberg, L. R., Rosi, F. D., and Herkart, P. G., in *Properties of Elemental and Compound Semiconductors*, edit. by Gatos, H. C., 25 (Interscience Pub., New York, 1960).
[2] Welker, H., *Technische Rundschau*, **50** (Nov. 30, 1956).
[3] Allen, J. W., *Nature*, **187**, 403 (1960).
[4] Gooch, C. H., Hilsum, C., and Holeman, B. R., *J. App. Phys.* (to be published).
[5] Bube, R. H., *J. App. Phys.*, **31**, 315 (1960).
[6] Blanc, J., Bube, R. H., and MacDonald, H. E., *J. App. Phys.*, **32**, 1666 (1961).

GEOLOGY

Natural Glass from beneath Red Clay on the Floor of the Atlantic

During April and May 1961 on a cruise of the R.V. *Chain*, a programme of coring deep-sea sediments from the Atlantic for geochemical investigations was conducted. At 19° 23′ N., 40° 53′ W. a modified Kullenberg corer was lowered through 4,430 m. of water to the summit of a rise on the ocean floor. The topography of the sea bed in this region is irregular, but between the hills and rises the average depth is approximately 4,900 m. At the point where the corer struck the sea bed the surface sediment is a red clay, but no sediment core was obtained since at a very short distance below the surface the red clay is underlain by solid material. The corer struck and broke the surface of this solid material, a fragment of which was recovered in the pilot corer, together with the overlying red clay. From the position of the solid fragment in the pilot corer the thickness of the clay can be estimated to be not more than 38 cm. The solid fragment is a very dark brown, almost black natural glass of irregular shape, but with approximate dimensions 3 cm. × 2·5 cm. × 1·3 cm. Two faces of the fragment were freshly broken surfaces. On two other faces a thin (1 mm.) zone of devitrification occurs with small areas of manganese deposition on the outer surface. The two other faces appear to be natural fractures. Internally the fragment contains small irregular cavities about which slight devitrifica-

tion has also occurred. When collected the fragment showed a marked tendency to disintegrate into smaller fragments, a consequence, no doubt, of the impact of the corer on the brittle glass. Red clay containing broken shards of the same glass was found adhering to the piston of the main corer, and it is likely, therefore, that the glass formation is too extensive to be interpreted as a boulder contained within the red clay. It is believed to represent the solid surface on which the overlying sediments rest. So far as is known this is the first sample collected of solid rock indisputably from below the sediments of the deep Atlantic.

The glass fragment when collected weighed approximately 18 gm. Part of it was crushed and natural glass fragments hand-picked from devitrified material for chemical analysis. Examined optically these glass fragments appear homogeneous without crystallites, microlites or any other crystallization phenomena. The chemical composition of this glass is quite remarkable, namely (percentages), SiO_2 25·81, Al_2O_3 19·35, TiO_2 2·02, Fe_2O_3 15·05, FeO 0·63, MnO 0·15, MgO 1·29, CaO 1·25, Na_2O 1·68, K_2O 0·89, H_2O 31·02, P_2O_5 0·08, Cl_2 0·97; uncorrected total 100·19 per cent, less 0 for Cl 0·22 per cent; corrected total 99·97 per cent. It is quite different from compositions of natural igneous glasses from the continental regions of the world. The high chlorine content suggests contamination with sea salts. However, the fragments analysed were hand-picked from the interior of the larger fragment collected, and such a high chlorine content, equivalent to 1·6 per cent sodium chloride, cannot be attributed to surface contamination by sea water during collection. The high water content also suggests contamination with sea water. Attempts to determine the distribution of the water between loosely bound positions (H_2O-) and tightly bound positions (H_2O+) did not meet with success. The loss in weight on heating the powdered glass for 20 hr. at 108° C. corresponded to 22·18 per cent of the total powder, but this figure slowly rises with continued heating. Loss of water appears to be accompanied by crystallization of the glass so that any H_2O- figure determined does not correspond to the original material. Thus the total H_2O figure has not been broken down into H_2O+ and H_2O-. The very high Fe^{+++}/Fe^{++} ratio suggests that the material was hydrated while still in a molten condition, supporting the view that the water content of this glass is original, despite being so much greater than the water content of silicate glasses produced experimentally in the laboratory.

A more extensive account of the properties of this glass and its possible origin will be published elsewhere. Meanwhile we urge oceanographers to make further attempts to collect samples from the subsediment surface of the Atlantic floor in order to demonstrate whether this first sample is truly representative of that surface or merely an unusual type of limited distribution.

Grateful acknowledgment is made to all aboard the R.V. *Chain* for help in the coring operations and particularly to R. G. Leahy, P. L. Sachs and G. L. Erlanger. This work was supported by the U.S. Office of Naval Research under a contract with the Woods Hole Oceanographic Institution.

G. D. Nicholls

Department of Geology,
University of Manchester.

V. T. Bowen

Woods Hole Oceanographic Institution,
Woods Hole, Mass.

Ultrabasic Liquids

For a number of years we have been investigating the problem of ultrabasic rocks with particular reference to evidence for and against the existence of ultrabasic liquids[1,2]. We believe that our published results have largely established their existence and this has been further substantiated by Wyllie[3], and by Clark and Fyfe[4]. Although Clark and Fyfe chiefly confine the application of their experimental results to what may be broadly termed the serpentinite problem, their conclusions have a very restricted validity if it is implied that serpentinites are the only type of ultrabasic rock which could have been mainly or wholly liquid. In the ultrabasic rocks investigated by us most of the olivine is not serpentinized.

The results of our recent investigation of picritic and peridotitic rocks add further support to the existence of ultrabasic liquids, but at the same time have revealed certain complicating factors the significance of which has yet to be evaluated. The principal purpose of this communication is briefly to record and discuss some of these results.

In West Greenland and the Scottish Hebrides we have made an extensive and detailed examination of marginal phenomena in what is by far the best developed series of ultrabasic (picrite and peridotite) minor intrusions within our knowledge. As recorded in ref. 2*a*, the degree of contact metamorphism is very variable and has no apparent relationship with the size of the intrusion. To the evidence of high temperature on Soay[2] we can now add similar evidence from the Island of Scalpay in Skye and from Ubekendt Ejland in West Greenland. On Scalpay, a picritic intrusion 3 ft. in average thickness has fused xenoliths of a very quartzose sandstone to a clear glass (Fig. 1). The picritic intrusion at Igdlorssuit on Ubekendt Ejland, where it is 10–12 ft. thick, has largely liquefied the marginal 3–6 in. of the contiguous zeolitic olivine-rich, basaltic lava (Fig. 2), the liquefaction possibly having been facilitated by the fluxing action of water derived from zeolites. But in the case of many otherwise similar ultrabasic minor intrusions, and in parts of the Soay, Scalpay and Igdlorssuit intrusions already mentioned, the effects of contact metamorphism are so slight that much lower temperatures are apparently implied. One can only infer that in such cases some factor other than the inward diffusion of water, suggested by Clark and Fyfe, has been responsible for the relative lack of thermal metamorphism. In seeking an explanation, it seems reasonable to refer to the manner and mechanism of emplacement of these intrusions. The differences between their contact effects may in fact be due not

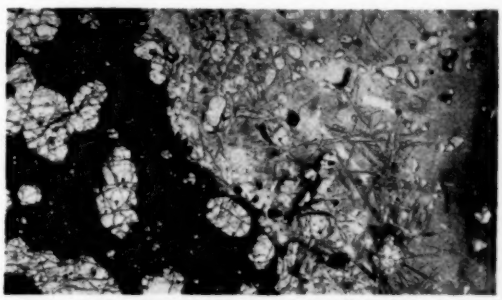


Fig. 1. An ultrabasic contact (containing olivine phenocrysts) with a sandstone xenolith (containing small quartz relicts and acicular orthopyroxene) largely fused to form a clear glass. (× 10)

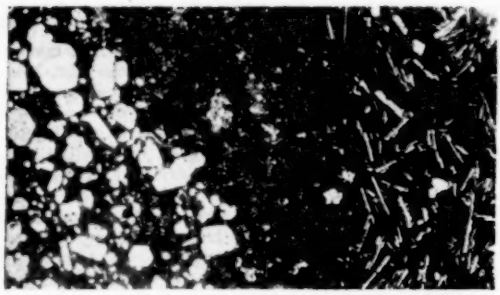


Fig. 2. A later ultrabasic contact (containing non-skeletal olivine pheno-crysts) with rheomorphic, fused lava mixed with an earlier ultrabasic contact facies (containing skeletal olivines). (× 3)

to differences in the temperature of the intruding magma, but to differences in the period of time during which the adjacent country rocks were maintained at similar temperatures; the total heat effect at any one point being dependent, among other things, on the amount of magma that has passed that point and its rate of movement. Variation within individual intrusions cannot be explained on this basis, but if ultrabasic magma, intruded at high temperature at one point, cooled and partially crystallized there before being translated to another position in relation to the country rock, it might well have much less thermal effect in its new position.

Variability in the ultrabasic contact facies might be explicable by mechanisms similar to the above. Development of skeletal olivines in the selvages of some intrusions, but not in others, could be accounted for by differences in rate of flow of magma. In cases, such as those on Ubekendt Ejland (where ultrabasic selvages in which all the olivines are skeletal give place when traced laterally to selvages in which the olivines are predominantly non-skeletal), the second mechanism, namely cooling and partial crystallization at one point followed by translation to another, could apply. In this way, the original, relatively quickly congealed selvages with skeletal olivines could be dispersed and replaced by selvages characterized by non-skeletal phenocrysts (Fig. 2). This does not diminish the significance of the development of skeletal olivines, which constitutes a much more common characteristic of ultrabasic contact facies than formerly suspected[5], as evidence of an ultrabasic liquid. Nor does it imply that, where the marginal olivine is not skeletal, the degree of contact metamorphism should always be appreciably less.

The direct experimental determination under hydrothermal conditions, by Clark and Fyfe, of the liquidus temperature of serpentinite, reinforces earlier deductions[3,6] which placed such temperatures considerably lower than had hitherto been regarded as possible. An extension of this work should yield experimental results more closely applicable to the wealth of natural data now available.

H. I. Drever
R. Johnston
C. M. Thomas

Department of Geology,
University, St. Andrews.

[1] Drever, H. I., *Medd. om Grønland*, **137**, 4 (1956).
[2] Drever, H. I., and Johnston, R., *Trans. Roy. Soc. Edin.*, **63**, 289 (1957); 2*a*, **63**, 459 (1958).
[3] Wyllie, P. J., *Min. Mag.*, **32**, 459 (1960).
[4] Clark, R. H., and Fyfe, W. S., *Nature*, **191**, 158 (1961).
[5] Drever, H. I., *Arctic, J. Arctic Inst. N. Amer.*, **11**, 199 (1958).
[6] Wyllie, P. J., *J. Petr.*, **2**, 1 (1961).

METALLURGY

Fatigue under Random Loads

It is often necessary to deduce the endurance of metals under a random succession of loads of varying magnitude from the behaviour of specimens tested with stress cycles of constant amplitude. One way of doing this is to use the cumulative damage law, which may be written:

$$N\sum_i p_i/N_i = 1 \qquad (1)$$

where N is the number of cycles of random loading required to produce fracture, p_i is the fraction of these cycles with stress amplitude s_i, and N_i is the endurance of a specimen tested at a constant stress amplitude equal to s_i.

This equation can be dangerously misleading. Thus Freudenthal and Heller[1] tested specimens of aluminium alloy and of steel using up to six levels of stress applied in random succession. The values of p_i were adjusted to give an exponential law $p_i \sim \exp(-h\,s_i)$ like that which describes the distribution of stresses in an aircraft from atmospheric gusts. Freudenthal and Heller found life-times in this test about six times shorter than those given by equation (1). They proposed a more elaborate semi-empirical formula including interaction effects between the damage produced at the different stress-levels. It seemed worth while to attempt a solution of this problem on the basis of the fatigue model proposed by me in previous communications[2,3].

According to this model notches are formed in the surface of the metal by randomly distributed slip. The fraction f of notches which have depths between z and $z + \mathrm{d}z$ after t cycles is given by:

$$f = F\exp(-z/\alpha t)$$

where F is a slowly varying function of z and t, and α is a constant. The total number of notches with depth greater than a critical value of z, say Z, is given by integration and will be of the form:

$$A\exp(-Z/\alpha t)$$

where A is another slowly varying function, and has a large numerical value. Now in a test at constant stress amplitude we suppose that all notches deeper than some fixed value of Z are liable to give rise to cracks, so that the probability of crack production per cycle can be written:

$$PA\exp(-Z/\alpha t)$$

where P is a constant. These cracks grow rapidly and soon lead to failure so that this expression can be taken as a mortality-rate for the specimens. It is very small until t becomes of the order $Z/K\alpha$, where K is $\log_e(PA)$ and is taken to be about 15.

Now suppose that tests are carried out at a series of different constant stress-levels. Both α and Z will depend on the value of the cyclic strain ε. As in a previous communication[3] we take α to be proportional to ε, and Z to be inversely proportional to ε. Thus we put $\alpha_i = a\varepsilon_i$ and $Z_i = c/\varepsilon_i$, where a and c are constants. Then the life-time of the specimen in a test at constant cyclic strain ε_i is given by:

$$N_i\,\varepsilon_i^2 = c/(aK) = C^2$$

in accordance with Coffin's relation between the cyclic strain and the endurance[4].

Now we consider the case of a random succession of cycles with a fraction p_i having cyclic strain ε_i. The

law of growth of the notches will be essentially the same as in tests at constant ε except that the constant α must be replaced by an average value $\bar{\alpha} = a\sum_i p_i \varepsilon_i^{\alpha}$.

Thus the total number of notches of depth greater than Z after t cycles will be given by:

$$A \exp\left(-Z/\bar{\alpha}t\right)$$

Now consider the probability that any fatigue cycle will generate a crack. This is given by the product of the fraction p_i of cycles with strain value ε_i and the number of notches with depth greater than $Z_i = c/\varepsilon_i$. Hence the mortality-rate will now be:

$$AP\sum_i p_i \exp\left(-Z_i/\bar{\alpha}t\right) \qquad (2)$$

and the life-time is determined by the value of t at which this sum ceases to be very small.

In order to apply this relation to Freudenthal and Heller's tests we assume the validity of Coffin's law in order to obtain values of ε_i from the life-times N_i. This leads to $\bar{\alpha} = a\,C\,\sigma$, where σ is written for the sum:

$$\sum_i p_i / N_i^{1/2}$$

Similarly we can write $Z_i = (c/C)\,N_i^{1/2}$. Substituting these in the exponential part of (2) gives $\exp(-KN_i^{1/2}/\sigma t)$ bearing in mind that $c/(aK) = C^2$. Now for each term of the sum in (2) there is a value of t at which this term becomes large. This value of t is given by $t_i = N_i^{1/2}/\sigma\ q_i$, where I have written q_i for $1 + (1/K)\log_e p_i$.

Of the various t_i values, the least, which we call t_m, is the one which determines the life-time, because the corresponding term in (2) is the first to become large. Thus on this theory the growth of the notches is determined by a mean value of α in which (in any event for the distributions used by Freudenthal and Heller) the low stress values are predominant. The critical depth Z on the other hand, and therefore the point at which a notch becomes a crack, is determined by one of the stress-levels, usually a high one.

The details of the calculation for one of Freudenthal and Heller's runs are shown in Table 1. This is the first of their runs with aluminium alloy specimens. The s_i values are given as fractions of the ultimate tensile strength, and the N_i values are taken from the smoothed curve and formulæ given by Freudenthal and Heller. It will be seen that whereas the lowest value of s makes the largest contribution to σ, the largest value of s is that which gives the smallest t_i, and may therefore be supposed to be responsible for the ultimate failure of the specimen. The experimental value of the life-time (Freudenthal and Heller's V_r') is $3{\cdot}76 \times 10^6$, in quite good agreement with the smallest value of t_i, namely, $4{\cdot}27 \times 10^6$. The life-time predicted by use of equation (1) is $1{\cdot}49 \times 10^7$, which is thus grossly over-optimistic.

Table 1. CALCULATION OF LIFE-TIME
(Run 22—aluminium alloy)

s_i	$N_i^{1/2}$	P_i	P_i / $N_i^{1/2}$		q_i		t_i	
0·35	34,000	0·822	2·42	10^{-5}	0·99	390	10^6	
0·45	5,750	0·1456	2·53		0·87	76		
0·55	1,150	0·02664	2·32		0·76	17·35	10^6	
0·65	490	0·00458	0·94		0·64	8·78		
0·75	255	0·0010	0·39		0·54	5·41		
0·85	158	0·000182	0·11		0·426	4·27		$= t_m$
			$\sigma =$ 8·71	10^{-5}				

Similar calculations have been carried out for the other runs on aluminium alloy specimens. The results are shown in Fig. 1, where the experimental life-time V_r' is plotted against the value of t_m. The mean value of the ratio V_r'/t_m is 0·85 with a standard deviation of 0·15 or 18 per cent, and the straight line in Fig. 1 is drawn to correspond to this ratio.

The corresponding calculations for Freudenthal and Heller's runs on steel specimens are complicated by the existence of a fatigue limit. These results, and the application of the theory to continuous distributions of other forms such as the Gaussian noise source used by Head and Hooke[5], will be published later.

A. N. MAY

Brooklyn Crystallographic Laboratory,
339 Cherry Hinton Road,
Cambridge.

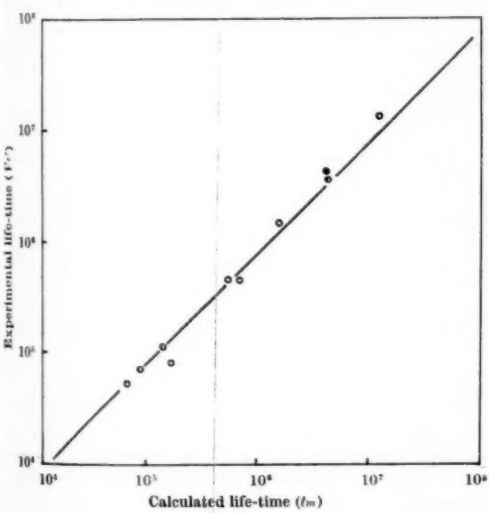


Fig. 1

[1] Freudenthal, A. M., and Heller, R. A., *J. Aero/Space Sci.*, **26**, 431 (1959).
[2] May, A. N., *Nature*, **185**, 303 (1960).
[3] May, A. N., *Nature*, **188**, 573 (1960).
[4] Coffin, L. F., in *Internal Stress and Fatigue in Metals*, edit. by Rassweiler, G. M., and Grube, W. L., 363 ((Elsevier, 1959).
[5] Head, A. K., and Hooke, F. H., *Intern. Conf. on Fatigue in Metals*, 301 (Institute of Mechanical Engineers, London, 1956).

CHEMISTRY

Formation of Active Nitrogen using Sources of Alternating Current and Direct Current

ACTIVE nitrogen has been generally investigated at pressures of several tenths of an mm. The majority of workers have used either a condensed or electrodeless discharge to produce the active species[1]. These two methods have been favoured to such an extent that little has been done to investigate other possibilities. However, work on the active gas at pressures above several mm. requires more efficient methods of production.

In 1932, Kaplan[2] obtained active nitrogen by using a 1 kW., 25,000 V., alternating current source and inferred that this was the first time that the after-glow was obtained by means other than a condensed discharge. More recently, Stanley[3] produced the active gas with a 10,000-V. alternating current supply in a capillary arc and found that the after-glow obtained at high pressures (about 500 mm.) differed markedly from the usual low-pressure spectrum. Berkowitz

et al.[4] used a source of alternating current to investigate the nitrogen after-glow mass-spectrometrically.

In the work reported here, part of an investigation of the chemical species produced by low-temperature plasmas, active nitrogen has been produced at 50 mm. by both uncondensed alternating current and low-voltage direct current sources. The equipment, similar to that described by Dehmelt[5], consisted of a conventional glass vacuum system and a 25-mm. internal diameter quartz discharge tube in which a 3/32 in. diameter tungsten cathode and 1/8 in. diameter tungsten anode were mounted with a 30-mm. gap. The effluent from the discharge tube was pumped down a decay tube 1 m. long and fitted with quartz windows for spectrographical observation. A 5-kW., 600–1,300 V. alternating current and 1 kW., 0–100 V. direct current source were connected in series across the electrodes. The alternating current was used to initiate the plasma, which was then sustained by direct current alone operating at 50–60 V. and 2–2·5 amp. A 0·8-Henry choke limited the alternating current to 4 amp.; the alternating current discharge was first induced with the aid of a Tesla coil. Argon was used as the main plasma medium with 1–2 per cent nitrogen added through a capillary leak. Commercial cylinder nitrogen (Linde) was used directly without additional purification.

At pressures greater than about 1 mm., it was observed that the characteristic yellow after-glow of the first positive system of nitrogen, the $B^3\Pi - A^3\Pi$ interaction, was produced when either the alternating current or direct current arc was operating. The identity of the after-glows thus produced was first established with a direct-vision spectroscope and then confirmed with the aid of a prism spectrograph. For purposes of comparison, Fig. 1 shows spectrograms of the after-glow produced by the two different sources under identical conditions of flow, pressure, etc. The spectrograms were taken with a small quartz Hilger; the Kodak *I–F* plate was calibrated with mercury lines which appear as the middle spectrum in Fig. 1. In the top and bottom spectra, the two features at the extreme right are the 11,7(5804 Å.) and 11,8(6251 Å.) bands of the first positive system of nitrogen. The remainder of the bands belong to the $v' = 0$ progression of the NOβ ($\beta^2\Pi - X^2\Pi$) interaction. No γ-bands of nitric oxide impurity are detectable in the spectrogram.

Several observations can be made regarding the spectra in Fig. 1. The direct current power input of approximately 150 W. produces a nitrogen spectrum at least several times as intense as an alternating current input of 3,000 W. Since the intensity of the after-glow is proportional to the square of the concentration of nitrogen atoms, the supply of direct current appears to be at least 50–70 per cent more effective in dissociating nitrogen than a source of alternating current twenty times as energetic. This tremendous difference in efficiency was checked in subsequent experiments by titration of the two streams of nitrogen atoms with nitric oxide[6]. With nitric oxide injected into the after-glow through a capillary leak, the ratio of pressures required to reach an end-point for direct- and alternating-current was 1·46. Under the conditions of these experiments, the flow-rate of nitric oxide varies at least with the square of the fore-pressure[7], so that the concentration of nitrogen atoms in the direct current after-glow was at least twice that in the alternating current after-glow. The low-pressure plasma can thus be regarded as an extremely efficient source of active nitrogen at pressures above 1 mm.

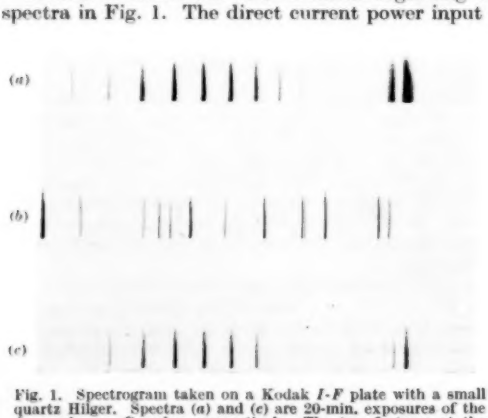


Fig. 1. Spectrogram taken on a Kodak *I-F* plate with a small quartz Hilger. Spectra (*a*) and (*c*) are 20-min. exposures of the d.c. and a.c. after-glow, respectively. The two features at the extreme right are first positive bands of nitrogen; the remaining bands belong to the NOβ system. Line (*b*) is the mercury spectrum used in calibrating the plate

This work is being carried on with financial support from the Battelle Memorial Institute.

G. G. Mannella

Department of Chemical Engineering,
Battelle Memorial Institute,
Columbus, Ohio.

[1] Mitra, S. K., *Active Nitrogen—A New Theory* (Indian Association for the Cultivation of Science, Calcutta, 1945).
[2] Kaplan, J., *Phys. Rev.*, **42**, 807 (1932).
[3] Stanley, C. R., *Proc. Phys. Soc.*, **67**, A, 821 (1954).
[4] Berkowitz, J., Chupka, W. A., and Kistiakowsky, G. B., *J. Chem. Phys.*, **25**, 457 (1956).
[5] Dehmelt, H. G., *Phys. Rev.*, **99**, 527 (1955).
[6] Harteck, P., Reeves, R. R., and Mannella, G., *J. Chem. Phys.*, **29**, 608 (1958).
[7] Moore, W. J., *Physical Chemistry*, 173 (Prentice-Hall, Inc., New York, 1950).

Microwave Spectrum of Fluoro-Acetonitrile

Fluoro-acetonitrile (FCH_2CN) is a molecule of some structural interest which is well suited to microwave study, all nuclei being well removed from the molecular centre of gravity in a structure of considerable dipole moment. It should thus be possible to locate the atoms of the $—CH_2CN$ group accurately with respect to the mass centre, by the method of isotopic replacement, and hence also to determine the position of the fluorine atom. As part of such an investigation, we report an assignment of the spectrum of the principal isotopic form. Transitions of the *R*-branch corresponding to the *A*-component of the molecular dipole have been identified for $J = 0 \rightarrow 1$, $1 \rightarrow 2$, $2 \rightarrow 3$ and $3 \rightarrow 4$, between 9 and 38 Gc./s.; *Q*-branch lines for the same component, $J_{1,J} \rightarrow J_{1,(J-1)}$, have been assigned for $J = 6$, 9, 10, 11 and 12. Corresponding to the *B*-component of the dipole, lines of the type $J_{0,J} \rightarrow J_{1,(J-1)}$ have been measured for J values of 1–5.

Analysis leads to the following rotational and centrifugal distortion constants, in Mc./s.:

A	= 36,577·47 ± 0·10	D_J	= 0·0033 ± 0·0007
B	= 4,781·29 ± 0·02	D_{JK}	= −0·068 ± 0·003
C	= 4,339·75 ± 0·02	δ_J	= 0·00056 ± 0·00001

Nuclear quadrupole splittings due to nitrogen-14 are resolvable, and of approximately the anticipated magnitude. They are at present being more accurately measured, as are Stark effect displacements for evaluation of the dipole moment components.

Intensities of the lines should permit examination of further isotopic forms, and work on this is continuing in order to allow completion of the structure determination. It may be noted, however, that, if the $-CH_2CN$ group were assigned a structure close to that in methyl cyanide[1], the constants so far measured would require a carbon–fluorine distance of about 1·38 Å., and an angle FCC near 111°. The present results are thus compatible with a carbon–fluorine distance similar to that in methyl fluoride[2], and a carbon–carbon distance similar to that in methyl cyanide. Definite conclusions, however, must await further measurements.

We thank the Department of Scientific and Industrial Research for a research studentship awarded to one of us (B. E. J.).

B. E. Job
J. Sheridan

Department of Chemistry,
The University,
Birmingham, 15.

[1] Thomas, L. F., Sherrard, E. I., and Sheridan, J., *Trans. Farad. Soc.*, **51**, 619 (1955).
[2] Andersen, F. A., Bak, B., and Brodersen, S., *J. Chem. Phys.*, **24**, 989 (1956).

Rubeanic Acid as Stabilizer of Aqueous Solutions of Ascorbic Acid

Especially noteworthy among the stabilizers of ascorbic acid solutions are sulphydric organic derivatives such as glutathione, thiourea, methylthiouracyl, cysteine[1–3], 2-carbethoxy-mercapto-1-methylglyoxaline[2,3], dithioglycerin, thioglycolic acid[2], etc. On the other hand, acids have found application as agents reducing the rate of oxidation of ascorbic acid in solutions. Among these oxalic acid plays an important part. In 2 per cent and 1 per cent dilutions it is applied as a stabilizer of synthetic ascorbic acid in various solutions and of ascorbic acid in biological material.

This suggested the use of rubeanic acid for preventing oxidation of ascorbic acid in aqueous solution. Rubeanic acid is a diamide of thio-oxalic acid. We wanted to investigate the stability of 0·01 per cent aqueous solution of ascorbic acid in the presence of rubeanic acid.

Ascorbic acid solutions containing rubeanic acid were prepared, and the ascorbic acid content was assessed during storage. Moreover, the same solutions were subjected to the effect of oxidizing factors, such as irradiation with a quartz lamp or introduction of cupric ions, the ascorbic acid content being measured all the time. This was carried out by means of Tillmann's method, consisting in the reduction of 2,6-dichlorphenolindophenol. Table 1 shows results obtained in determining the stability of ascorbic in rubeanic acid solutions.

Table 1. Stability of 0·01 per cent Aqueous Solution of Ascorbic Acid in the Presence of Rubeanic Acid

Time of storage (hr.)	Ascorbic acid content (per cent of initial content) in aqueous solution	in 0·01 per cent rubeanic acid	in 0·001 per cent rubeanic acid
—	100·0	100·0	100·0
0·5	82·0	99·2	98·3
1·0	49·9	98·3	97·6
2·0	33·3	98·3	96·0
3·0	16·6	97·6	96·0
4·0	13·3	97·6	94·4
23·0	0·0	92·8	80·0

In Table 2 are assembled the results obtained in assessing the stability of ascorbic acid solutions irradiated with a quartz lamp.

Table 2. Stability of 0·01 per cent Aqueous Solution of Ascorbic Acid in the Presence of Rubeanic Acid on Irradiation with a Quartz Lamp

Time of storage (hr.)	Ascorbic acid content (percentage of initial content) in aqueous solution	in 0·01 per cent rubeanic acid
—	100·0	100·0
0·5	69·7	98·3
1·0	51·3	98·3
2·0	21·0	98·3
3·0	9·1	97·6
4·0	3·3	97·6

Table 3 shows the ascorbic acid content in 0·01 per cent and 0·001 per cent solutions of rubeanic acid in the presence of copper ions (0·001 per cent and 0·0001 per cent copper sulphate).

Table 3. Stability of Ascorbic Acid in 0·01 per cent Solution, in the Presence of Rubeanic Acid and on Admixture of Copper Ions

Time of storage (hr.)	Ascorbic acid content (percentage of initial content) in 0·001 per cent $CuSO_4$	in 0·01 per cent rubeanic acid: in 0·001 per cent $CuSO_4$	in 0·01 per cent rubeanic acid: in 0·0001 per cent $CuSO_4$	in 0·001 per cent rubeanic acid: in 0·001 per cent $CuSO_4$	in 0·001 per cent rubeanic acid: in 0·0001 per cent $CuSO_4$
—	100·0	100·0	100·0	100·0	100·0
0·5	10·0	99·0	99·3	98·3	100·0
1·0	0·0	99·0	99·3	97·4	99·2
2·0	—	98·1	98·4	91·2	98·3
3·0	—	95·8	96·5	91·2	96·6
4·0	—	93·4	95·9	91·2	95·8
24·0	—	80·0	86·3		
48·0	—			66·9	72·3

From the results in Tables 1–3 rubeanic acid is seen to be a stabilizer of ascorbic acid. The stability of the solutions is dependent on the amount of rubeanic acid added. Ultra-violet radiation and copper ions in solution, which are factors accelerating the oxidation of ascorbic acid, lose their activity in the presence of rubeanic acid.

Aleksandra Smoczkiewiczowa
Janina Grochmalicka

Institute of Inorganic and
Analytical Chemistry,
Medical Academy,
Poznań, Poland.

[1] Drevon, B., Nofre, C., and Cier, C., *Ann. Pharm. Franc.*, **16**, 495 (1958).
[2] Feldheim, W., and Seidemann, J., *Pharmazie*, **14**, 12 (1959).
[3] Feldheim, W., and Seidemann, J., *Pharmazie*, **14**, 80 (1959).

Ligand Field for Chromic Ions in the Corundum Lattice

Recent work on absorption spectra and lattice spacings in the Al_2O_3–Cr_2O_3 solid solution[1–5] has been in sufficient agreement to warrant a fitting of the optical results to the crystal field-splitting diagram[7]. The relevant terms are shown in Fig. 1, which also shows the transitions involved in the yellow and blue absorption bands.

Assuming the Racah parameter B to be constant throughout the system, it is impossible to obtain a fit no matter what value of B is chosen. An example is shown in Fig. 2, with $B = 650$ cm.$^{-1}$. This value was chosen to give a fit at about 5·5 per cent Cr_2O_3, where the observed maxima for ruby (Graham[1]) and $Cr(H_2O)_6^{3+}$ (Tsuchida[8]) are identical.

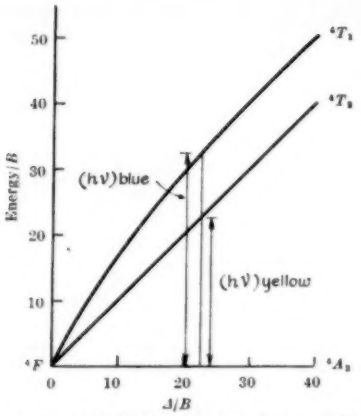


Fig. 1. Energy-level diagram for the splitting of ^4F in an octahedral field

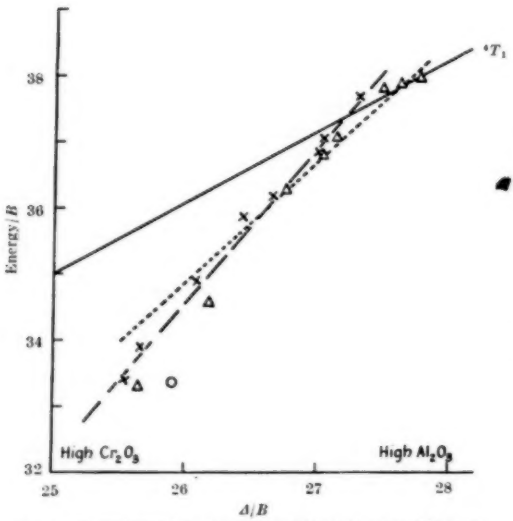


Fig. 2. Experimental energy values derived for $B = 650$ cm.$^{-1}$. ○, Graham; △, Schilly; ×, Schmitz-Dumont and Reinen; — —, Schultz

It is known that an increase in covalent bonding[9] and/or charge transfer[10] results in a decrease in B. There is evidence[1] for the existence of bonding other than ionic in both Al_2O_3 and Cr_2O_3, and it can be shown that the proportion of such bonds increases as the chromium content decreases. This follows from a consideration of cell volumes. Thus if we assume ionic bonding and regular octahedral surroundings for the Al^{3+} and Cr^{3+} ions in pure Al_2O_3 and Cr_2O_3 respectively, we would expect an increase in unit cell volume of 10·35 per cent in passing from Al_2O_3 to Cr_2O_3, whereas the observed increase is 13·5 per cent. The contents of the unit cell (on the basis of ionic volumes) have increased in volume by only 2·8 per cent, so that in the absence of other types of bonding one would expect distortions of the octahedron to accommodate a good deal of the increase calculated here. The observed increase in cell volume is therefore much greater than that to be expected unless Al_2O_3 is more covalent in character than Cr_2O_3. The lattice spacings[1,2] tend to confirm this conclusion, as the effective radius of the chromium ion is greater at the alumina-rich end of the solid solution, perhaps indicating a greater amount of charge transfer. Based on present ideas, it would be expected that B would increase as the chromium content increases.

However, if we calculate the values of B required to fit the experimental and theoretical curves at all points, we find that B increases approximately linearly with decreasing Cr_2O_3 content or increasing Δ (Fig. 3). Although there is some scatter between observers, the result is quite clear, and the variation of B is very large. The results of Graham and of Schultz are closely linear, and the lines of best fit have been plotted in Figs. 1–3. The accurate single crystal absorption data of Mandarino[4] on weighting the ordinary and extraordinary components in the ratio 2 : 3 according to the experimental result of Schultz[11] give a value of Δ 18·11 cm.$^{-1}$ for Al_2O_3 in excellent agreement with ref. 1.

The increase in Δ as the chromic oxide content decreases is easily explained on the basis of decreasing metal-ligand spacing, but the corresponding increase of B is anomalous. Since the axial ratio is almost constant throughout the system, the trigonal component of the field is unlikely to account for the anomaly.

Goodenough[12] points out that interactions between the ligands and the d-shell of the metal in the corundum structure are weak if there are three electrons or less in the d-shell, and postulates direct cation-cation coupling as the cause of antiferromagnetic ordering in these structures. Chromic oxide itself is antiferromagnetic with a Néel temperature near room temperature, and it would appear that the overlap of the d-orbitals of adjacent Cr^{3+} ions which results in the antiferromagnetic ordering may also explain the absorption spectrum if the d-shells interact to some extent even in the dilute solutions[13]. The decrease in B would then correspond to increasing cation–cation interaction as the chromium content increased. This is again consistent with the structural results[1], as the metal-to-metal distance in Cr_2O_3, 2·58 Å., is significantly less than that in Al_2O_3, 2·73 Å. In terms of the cation radius, the difference is even more marked.

It is felt that the anomalous values of B and Δ obtained by one of us (J. G.) for pure Cr_2O_3 (the separate points shown in Figs. 2 and 3) are associated with

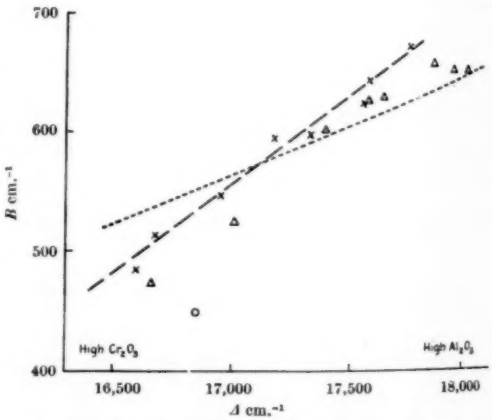


Fig. 3. Variation of B with crystal field (details as for Fig. 2)

antiferromagnetic ordering in this sample. This would be expected to result in a discontinuous decrease in B, but the accompanying increase in Δ is not so easy to understand.

J. GRAHAM
D. E. SCAIFE

Commonwealth Scientific and Industrial Research Organization, Box 4331, G.P.O., Melbourne.

[1] Graham, J., *J. Phys. Chem. Solids*, **17** (1/2), 18 (1960).
[2] Schultz, G., *Conf. Physics and Chemistry of Crystal Phosphors (Greifswald*, 1959), 219 (Akademie-Verlag, Berlin, 1960).
[3] Schmitz-Dumont, O., and Reinen, D., *Z. Elektrochem.*, **63** (8), 978 (1959).
[4] Mandarino, J. A., *Amer. Mineralogist*, **44** (9/10), 961 (1959).
[5] Jan, J. P., Steinemann, S., and Dinichert, P., *Helv. Phys. Acta*, **33** (2), 123 (1960).
[6] Neuhaus, A., *Z. Krist.*, **113**, 195 (1960) (includes spectra by W. Schilly).
[7] Tanabe, Y., and Sugano, S., *J. Phys. Soc., Japan*, **9**, 766 (1954).
[8] Tsuchida, R., see ref. 6, 775.
[9] Schäffer, C. E., and Jorgensen, C. K., *J. Inorg. Nuclear Chem.*, **8**, 143 (1958).
[10] Dunn, T. M., *J. Chem. Soc.*, (2) 623 (1959).
[11] Schultz, G., *Annalen der Phys.*, **6** (7/8), 345 (1960).
[12] Goodenough, J. B., *J. App. Phys.*, **31**, 3595 (1960).
[13] Schäffer, C. E., *J. Inorg. Nuclear Chem.*, **8**, 149 (1958).

BIOCHEMISTRY

Biosynthesis of L-Ascorbic Acid: Missing Steps in Animals incapable of synthesizing the Vitamin

IT has been reported[1] that cyanide greatly stimulates the synthesis of L-ascorbic acid from D-glucuronolactone by the liver microsomes of the rat. By this method using cyanide it was found that either the liver or the kidney tissue of all the species examined which are known to be independent of an exogenous source of ascorbic acid was able to synthesize L-ascorbic acid, while the corresponding tissue of those which are dependent on such a source could not. Whether the enzyme system concerned was present in the liver or the kidney tissue depended on the position of the species in the phylogenetic scale of evolution[2]. This technique revealed further that, apart from man, monkey and the guinea pig, the India fruit bat (*Pteropus medius*) and the red-vented bulbul (*Pycnonotus cafer*) were unable to synthesize L-ascorbic acid, and this was confirmed by the production of experimental scurvy in the bulbul and its cure by the administration of L-ascorbic acid[3]. This communication concerns the location of the genetic defect in all these species, which distinguishes them from those which can synthesize L-ascorbic acid.

According to a scheme given elsewhere[4] indicating the probable biosynthetic pathway of ascorbic acid in those species which can make it, the following representation (Fig. 1) may be made in regard to the substrates and the enzymes of the microsomes and of the soluble supernatant involved in this synthesis.

Fig. 1 indicates that with D-glucuronolactone as substrate, microsomal enzymes alone can convert it into L-ascorbic acid through L-gulonolactone and 2-keto-L-gulonolactone. With D-glucuronic acid as substrate, however, the microsomes require the addition of the soluble supernatant which supplies the necessary enzymes to convert D-glucuronic acid into L-gulonolactone, which can be then converted into L-ascorbic acid by the microsomes. We have previously reported[4] that the failure of the guinea pig

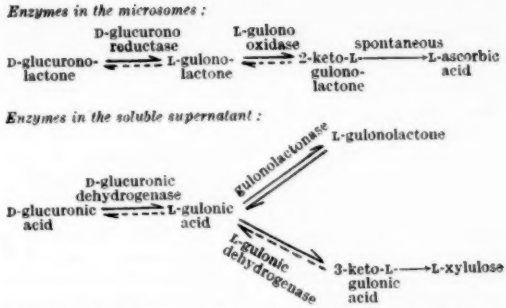


Fig. 1. Schematic representation showing the substrates and the enzymes of the microsomes and of the soluble supernatant involved in the biosynthesis of L-ascorbic acid in animal tissue

to synthesize L-ascorbic acid was due to the absence of L-gulono oxidase, which oxidizes L-gulonolactone to 2-keto-L-gulonolactone. Using the tracer technique, this was also observed by Burns *et al.*[5] and by Burns[6] in regard to man, monkey and the guinea pig.

It has now been found, however, that not only L-gulono oxidase but also the other microsomal enzyme, D-glucurono reductase, which converts D-glucuronolactone into L-gulonolactone, is specifically absent from those species which cannot synthesize L-ascorbic acid. This has been demonstrated by the fact that, under anaerobic conditions, incubation of D-glucuronolactone with liver or kidney microsomes from man, monkey, the bat, the guinea pig and the bulbul in the presence of cyanide did not result in the formation of L-gulonolactone whereas in similar experiments with microsomes from rat and goat livers, L-gulonolactone was produced as an intermediate. L-gulonolactone was identified by paper chromatography of the deproteinized and concentrated incubated mixture using: (*a*) ethyl acetate/acetic acid/water (3:1:3, v/v, upper phase; descending); (*b*) ethanol/acetic acid/water (8:1:1, v/v; descending); (*c*) acetone : water (4 : 1, v/v; ascending) as the irrigating solvents. The R_F values for D-glucuronolactone and L-gulonolactone were: (*a*) 0·51 and 0·43; (*b*) 0·62 and 0·54; (*c*) 0·72 and 0·55 respectively. The lactones were identified on the chromatogram by the hydroxamic acid method[4]. L-gulonolactone has also been identified by Suzuki *et al.*[7] as a product of anaerobic incubation of D-glucuronolactone with rat-liver microsomes in the presence of cyanide.

The absence of D-glucurono reductase was further revealed in the following way. The microsomes of the liver or kidney tissue (40 mgm. protein) from all the aforesaid species which cannot synthesize L-ascorbic acid were incubated with D-glucuronolactone (20 μmoles) in the presence of sodium phosphate buffer (0·02 *M*, *p*H 7·0) and potassium cyanide (0·05 *M*). After incubation, the mixture was deproteinized with perchloric acid, neutralized with caustic potash and, after filtering off potassium perchlorate, freed from cyanide by acidification with *N* hydrochloric acid followed by evaporation to dryness under suction. The residue was taken up in a minimum volume of water, neutralized and then incubated with goat liver microsomes, but no L-ascorbic acid was formed. Had there been any formation of L-gulonolactone, it would have led to the synthesis of L-ascorbic acid in the absence of cyanide on addition of goat liver microsomes[4]. A control experiment starting with L-gulonolactone (5 μmoles) instead of D-glucuronolactone

resulted in the formation of L-ascorbic acid (0·5 μmole) indicating little destruction of L-gulonolactone in the process of removal of cyanide.

In the scheme given (Fig. 1), the supernatant enzymes, converting D-glucuronic acid into L-xylulose and into L-gulonolactone, have been found to be present in all the above-mentioned species requiring dietary ascorbic acid[8]. Thus there are two genetic blocks in regard to the synthesis of L-ascorbic acid in these species: (1) the absence of D-glucurono reductase so that D-glucuronolactone cannot be reduced to the intermediate L-gulonolactone; (2) the absence of L-gulono oxidase so that L-gulonolactone cannot be oxidized to the intermediate 2-keto-L-gulonolactone, even if the enzymes of the soluble supernatant bring about the conversion of D-glucuronic acid into L-gulonolactone. That these two enzymes are actually present but are masked by some microsomal inhibitory substance is considered unlikely, as the addition of the microsomes from these species to the solubilized enzyme from rat liver, prepared as described before[4], was not found to inhibit the synthesis of L-ascorbic acid by the latter.

Our thanks are due to the Indian Council of Medical Research for financing this work.

I. B. Chatterjee
N. C. Kar
N. C. Ghosh
B. C. Guha

Department of Applied Chemistry,
University College of Science and Technology,
Calcutta, 9.

[1] Chatterjee, I. B., Ghosh, N. C., Ghosh, J. J., and Guha, B. C., *Sci. and Cult.*, **23**, 382 (1958).
[2] Roy, R. N., and Guha, B. C., *Nature*, **182**, 319 (1958).
[3] Roy, R. N., and Guha, B. C., *Nature*, **182**, 1689 (1958).
[4] Chatterjee, I. B., Chatterjee, G. C., Ghosh, N. C., Ghosh, J. J., and Guha, B. C., *Biochem. J.*, **74**, 193 (1960).
[5] Burns, J. J., Peyser, P., and Moltz, A., *Science*, **124**, 1148 (1956).
[6] Burns, J. J., *Nature*, **180**, 553 (1957).
[7] Suzuki, K., Mano, Y., and Shimazono, N., *J. Biochem.* (Japan), **47**, 848 (1960).
[8] Chatterjee, I. B., Kar, N. C., Ghosh, N. C., and Guha, B. C., *Ann. N.Y. Acad. Sci.*, **92**, 36 (1961).

Riboflavin and Liver Sulphydryl-Levels in Mice

During the course of a series of investigations of the sulphydryl content of mouse liver under varying experimental conditions, it was found that during a period of nine months there was a steady increase in the level found in control animals. The liver —SH content rose from 31·4 ± 1·7 μgm. per 100 mgm. wet weight to 34·3 ± 5·9 μgm. per 100 mgm. wet weight. In all cases the groups of animals used comprised a minimum of 10 Strong *A* male mice. This steady increase in control-levels was succeeded by a sharp fall to 27·7 ± 2·6 μgm. per 100 mgm. wet weight in a group of similar animals. Consideration of factors which might have been involved in this variation led to suspicion of the diet, particularly since, during the period in question, growth-rates in these animals had shown deviations from the expected pattern. Further examination of the diet question focused suspicion on the vitamin content, and especially on the constancy of the riboflavin-level. We were thus led into an investigation of the influence of dietary riboflavin on liver —SH levels.

All measurements of —SH levels recorded in the following paragraphs have been made by the method described by Calcutt and Doxey[1]. Whereas in the original use of this technique millivoltmeters were used for the potentiometric titrations these have now been replaced by transistorized titrimeters based on Stock's design[2]. These instruments have proved superior to millivoltmeters in operation as the settling time is negligible and scale movements are large and easily read.

All experimental work has been carried out with Strong *A* mice. These have been used in batches of the same age and, so far as possible, of approximately equal weights. This latter precaution has been taken in view of Calcutt's[3] finding of an inverse relationship between body weight and —SH content per unit mass of liver. The effects of addition and withdrawal of riboflavin from the diet have been examined and also the effects of riboflavin given by injection.

Addition of riboflavin to drinking water. Twenty mice were divided into two groups of ten each. While both groups were maintained on a normal pellet diet, one group received fresh water to drink whereas the second was given a saturated solution of riboflavin in tap-water. After four days the liver —SH content was estimated for all the animals. The group which had been given water had an —SH level of 33·9 ± 2·8 μgm. per 100 mgm. of wet weight of liver, while the corresponding figures for the group given riboflavin were 33·5 ± 2·6 μgm. of —SH per 100 mgm. of wet weight of liver. There is obviously no significant difference between the two groups. Since the solubility of riboflavin is only 0·01 per cent in water, the intake by the mice from this source would be small. Further, the actual absorption may have been even smaller, as mice eat and drink at separate times, and the evidence collected by Bro Rasmussen[4] indicates a requirement for the presence of protein for the adequate absorption of riboflavin. The slightly bitter taste of the solution may also have limited drinking by the mice in question. In the circumstances, the results obtained in this experiment are not surprising.

Withdrawal of riboflavin from the diet. Thirty male mice were placed on a synthetic diet obtained as a vitamin-free diet from British Drug Houses, Ltd., of London. To this, vitamins were added in the amounts used by Greenstein, Otey, Birnbaum and Winitz[5] in their experiments on synthetic diets. After 28 days, during which period the animals maintained their weight and health, ten were taken as controls and the liver —SH content was estimated. The remaining animals were transferred to the vitamin-free diet supplemented, as previously, with all vitamins except riboflavin. These animals were killed in batches of five at intervals for liver —SH measurements. The results are shown in Fig. 1, where it will be seen that the liver —SH content rose above the control-level seven days after withdrawal of the riboflavin.

Effects of riboflavin by injection. Thirty male mice were divided into two batches of 15 each. One lot received an intraperitoneal injection of 0·1 mgm. of riboflavin in 1 ml. of distilled water each mouse. Liver —SH content was estimated on all animals, those that had received riboflavin being killed 1 hr. after the injection. The control animals had 34·5 ± 4·2 μgm. of —SH per 100 mgm. of wet weight of liver whereas the corresponding figure for the riboflavin-treated animals was 28·8 ± 4·75 μgm. of —SH. In a similar series, using female mice, the treated animals were killed 18 hr. after the injection. This time the control —SH level was 38·6 ± 3·8 μgm. per 100 mgm. wet weight of liver and the level in the treated animals was 25·3 ± 5·0 μgm. per 100 mgm. wet weight of liver. As a further experiment a batch of male mice was injected, as previously, with an

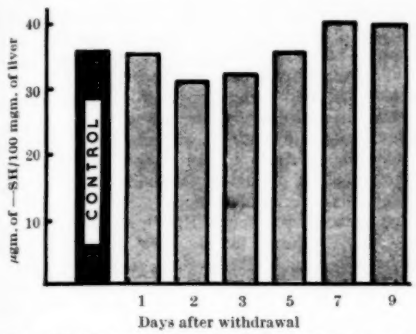


Fig. 1. Effects of withdrawal of riboflavin from mouse diet on liver —SH levels

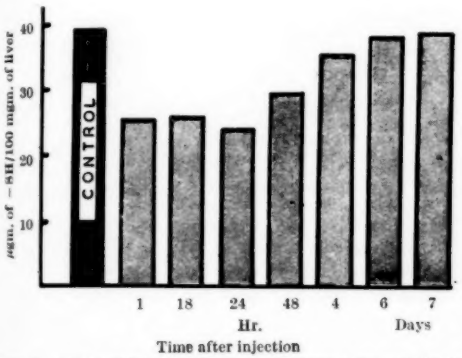


Fig. 2. Effects of injected riboflavin on mouse liver —SH levels

aqueous solution of riboflavin. These were killed in batches at daily intervals and the liver —SH level estimated. Return to the control-level did not take place until six days after treatment.

Tissue riboflavin-levels have often been associated with tissue protein content. As a follow-up to the above experiments, which were performed on mice fed a high-quality diet, the effects of riboflavin on animals on an inferior diet have been examined. Mice were fed rice and water only for ten days and then received an injection of 0·1 mgm. of riboflavin in 1·0 ml. of distilled water. Estimates of liver —SH level showed that this remained depressed until six days after treatment, as in the previous experiment. The results are shown in Fig. 2.

It is known that riboflavin plays a part in oxidation/reduction systems as a consequence of its participation in various enzyme systems. The experimental results reported here now indicate that riboflavin may exercise a function in relation to the general oxidation/reduction status of the liver cell. This activity would appear to be independent of the nutritional state of the animal, since there is no apparent distinction between the effect of injected riboflavin in well-fed or poorly fed animals. From this it seems unlikely that the effect involves the riboflavin in conjunction with the proteins of the cell.

The actual mechanism of the effect is at present unknown. Iwaki[6] has shown that a direct combination of glutathione and riboflavin can occur, but even if this did happen the linkage would probably be broken by the more reactive mercurial used in the —SH estimation. Riboflavin can act as an electron acceptor, and it is possible that, rather than any direct chemical combination of the vitamin with cellular components, we have an induced oxidation of various cellular components which, in turn, are responsible for maintenance of sulphur-containing groups in the reduced form. It has in fact been suggested by Booth and Boyland[7] that riboflavin inhibits the microsomal hydroxylating system by causing the oxidation of reduced triphosphopyridine nucleotide, which is required for maintenance of —SH in the system.

Recently, Hsu and Chow[8] have found that thiamine deficiency in rats leads to an elevation in the level of liver glutathione. This finding, in conjunction with the results recorded above, suggests that dietary vitamin-levels, at least of thiamine and riboflavin, may take a major role in determining the biochemical status of the liver. Further, it once more stresses the extreme importance of strict dietary control of animals used for biochemical work.

G. Calcutt
D. Doxey
Joan Coates

Department of Cancer Research,
Mount Vernon Hospital and
the Radium Institute,
Northwood, Middlesex.

[1] Calcutt, G., and Doxey, D., *Exp. Cell Res.*, **17**, 542 (1959).
[2] Stock, J. T., *Analyst*, **83**, 56 (1958).
[3] Calcutt, G., *Nature*, **186**, 84 (1960).
[4] Bro Rasmussen, F., *Nutrition Abst. and Revs.*, **28**, 1 and 369 (1958).
[5] Greenstein, J. P., Otey, M. C., Birnbaum, S. M., and Winitz, M., *J. Nat. Cancer Inst.*, **24**, 211 (1960).
[6] Iwaki, G., *J. Physiol. Soc., Japan*, **13**, 351 (1951), quoted in *Chem. Abst.*, **46**, 1606g (1952).
[7] Booth, J., and Boyland, E., *Biochem. J.*, **70**, 681 (1958).
[8] Hsu, J. M., and Chow, B. F., *Proc. Soc. Exp. Biol.*, **104**, 178 (1960).

In vivo and *in vitro* Inhibition of Aromatic Hydroxylation by Riboflavin

Detailed and extensive investigations of the metabolism of polycyclic hydrocarbons have been carried out over many years, but it is only recently that information linking the metabolism of these agents with an enzymic system present in liver has become available. It has been found that hydroxylation of the carcinogenic 3:4 benzpyrene[1] and the non-carcinogenic naphthalene[2] is brought about by an enzyme, or 'hydroxylase'[1], which occurs in the microsomal fraction of liver homogenates, this system requiring reduced triphosphopyridine nucleotide and molecular oxygen for activity. One of us (K. H. H.) has found that the same or a similar system is also active against pyrene and 1:2 benzanthracene. The products obtained are similar to those obtained during *in vivo* metabolism. This work will be reported in detail elsewhere.

This hydroxylating system is susceptible to sulphydryl complexing agents, such as *p*-chloromercuribenzoic acid. The information concerning the effects of riboflavin on liver sulphydryl-levels reported by Calcutt, Doxey and Coates (see previous communication) led us to further consideration of the effect of riboflavin on microsomal hydroxylation. Previously, it had been found by Booth and Boyland[2] that flavine mononucleotide (FMN) is an effective inhibitor of the microsomal hydroxylation of naphthalene. It seemed desirable, therefore, to see whether riboflavin also affected hydroxylation of other compounds.

The pyrene hydroxylating system (active in the conversion of pyrene to 3-hydroxypyrene) was chosen for the first investigation. The low solubility

Table 1. Inhibition of Pyrene Hydroxylation by Riboflavin

Compound	Concentration	Percentage inhibition
'Beflavit'	10^{-3}	100
	10^{-4}	95
'Dummy' 'Beflavit'	10^{-3}	70
	10^{-4}	15
FMN	10^{-3}	60
	10^{-4}	20

of riboflavin in aqueous solutions led us to the use of a concentrated (5 mgm./ml.) commercial preparation made in a solvent base. This was 'Beflavit', prepared by Roche Products, Ltd., England. For control purposes the solvent base preparation was also used. The results, together with those obtained using FMN, are summarized in Table 1.

It is evident from these figures that the solvent base itself contributes to the inhibitory powers of 'Beflavit', but even when allowance is made for this, riboflavin itself appears appreciably more active than the FMN.

Identical findings were then obtained in a similar series of experiments based on the metabolism of 3 : 4 benzpyrene.

The effect of riboflavin on the hydroxylation of 3 : 4 benzpyrene *in vivo* was then examined. Ten mice were each given an intraperitoneal injection of 0·1 mgm. of riboflavin in water and, 1 hr. later, an intravenous injection of 0·6 mgm. of benzpyrene as a colloid in 1 ml. of water. A second group of ten mice each received a similar benzpyrene injection. This group acted as controls. The excretion of phenolic metabolites, predominantly 8-hydroxybenzpyrene, in the fæces was then followed over a period of 48 hr. The animals treated with the hydrocarbon alone excreted approximately 25 per cent of the total injected dose as phenolic derivatives during this period. The difference between this group and that which had received prior treatment with riboflavin was striking, for this group excreted less than 1 per cent of the dose as metabolites during the same period. The benzpyrene in this group was not excreted unchanged, since only trace amounts were detected in the urine and fæces. Whether it is stored or metabolized in a different manner is still to be determined.

This last experiment provides excellent confirmation of the *in vitro* experiments and also supports the view that the microsomal hydroxylating system observed *in vitro* is a true representation or model of the normal cellular process. Further, this last experiment is interesting in that this is the first time that it has been shown that the metabolism of benzpyrene may be influenced by dietary or other external agents.

It is not yet clear which part of the hydroxylating system is inhibited by riboflavin. It appears unlikely that riboflavin becomes bound to the enzyme system, since microsomes prepared from animals pretreated with riboflavin show undiminished activity as compared with control preparations, provided that they are washed several times with 1·15 per cent potassium chloride solution. This would suggest that the effect is on the coenzyme moiety rather than the enzyme itself.

Apart from the more obvious implications arising from this work in respect of the nutritional state of animals used in metabolic studies, there is the question of riboflavin and carcinogenesis by polycyclic hydrocarbons. Boyland[3] has given reasons for considering that carcinogenesis by polycyclic hydrocarbons is associated with the metabolism of these compounds. If this is true, then the influence of riboflavin on the metabolism of these agents should be reflected in the carcinogenic activity. Further evidence of possible effects of riboflavin in carcinogenesis is given in the finding that the hepatocarcinogen (4′ dimethylamino-azobenzene) is metabolized by the same, or a similar, microsomal enzymic system[4], whereas the carcinogenic action of this agent is inhibited by a high dietary riboflavin intake[5]. Further consideration of these problems will be given elsewhere.

We are grateful to Roche Products, Ltd., Welwyn Garden City, England, for the gift of samples of the solvent base in which 'Beflavit' is prepared.

K. H. Harper
G. Calcutt

Nutritional Research Unit,
Huntingdon, and
Department of Cancer Research,
Mount Vernon Hospital
and the Radium Institute,
Northwood, Middlesex.

[1] Conney, A. H., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, **228**, 753 (1957).
[2] Booth, J., and Boyland, E., *Biochem. J.*, **70**, 681 (1958).
[3] Boyland, E., in *Biochem. Soc. Symposium* No. 5, edit. by Williams, R. T. (Camb. Univ. Press, London, 1950).
[4] Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P., *Science*, **93**, 308 (1941).
[5] Conney, A. H., Miller, E. C., and Miller, J. A., *Cancer Res.*, **16**, 450 (1956).

Effect of Insulin on the Phosphorus-32 Mononucleotide Labelling of the Isolated Rat Diaphragm in the Controlled Absence of Extracellular Glucose

Extensive work on glucose transport into the cell under the influence of insulin and on the metabolic consequences of this transport has been carried out on the eviscerated animal[1], the isolated rat diaphragm[2], the isolated perfused heart[3] and the isolated epididymal fat pad[4,5]. Insulin stimulation seems to involve not only an enhanced transport-rate, but also an increased intracellular distribution space as measured with non-metabolizable sugars[2] and amino-acids[6,7]. However, some recent reports[8,9] dealing primarily with incorporation of carbon-14 into proteins of rat diaphragm, in the absence of added glucose, demonstrated a striking insulin effect, which could not be explained by an increased accumulation of amino-acid precursors of the labelled protein. This effect may be correlated[10] with another report mentioning the action of insulin on endoplasmic reticulum proliferation in the fat pad[11]. Whatever the mechanism of action of insulin may be, stimulation of glucose transport, protein synthesis or any other metabolic event is bound to be reflected in the turnover of free mononucleotides and especially adenosine nucleotides, the role of which is essential in the activation of sugars, fatty acids[12] and amino-acids[13].

The present communication reports an effect of insulin on the labelling of free mononucleotides in the isolated rat diaphragm in the controlled absence of extracellular glucose. The isolated rat diaphragms were first washed, for 5 min., in Krebs–Ringer bicarbonate medium[14] at 37°, in an atmosphere of 95 per cent oxygen plus 5 per cent carbon dioxide. After removal and careful blotting of the diaphragms, they were incubated in fresh Krebs–Ringer bicarbonate medium with or without added glucose and insulin.

Table 1. Influence of Insulin on the Labelling of Phosphates in the Isolated Diaphragm with or without Glucose (Normal Rat)

	μgm. phosphorus/100 mgm. fresh weight				Specific activity (c.p.m./μgm.) (percentage extracellular or intracellular phosphorus)			
Glucose (2 mgm./ml.)	0	0	+	+	0	0	+	+
Insulin	0	+	0	+	0	+	0	+
Extracellular phosphorus	1·2	1·1	0·9	0·9	20,200	21,600	27,100	25,300
Intracellular phosphorus	23·3	28·6	24·6	28·6	1,540	1,235	1,835	1,405
					% E 7·7	5·7	6·8	5·6
P.C.	15·8	12·9	20·2	17·7	634	799	869	979
					% I 41	67	47	70
ATP	30·5	31·1	32·5	23	~ 421	602	699	734
					% I 27	49	38	52
ADP	7·8	7·8	5·9	7·9	~ 289	452	354	540
					% I 19	37	19	38
U + G	3·9	3·6	2·5	2·5	~ 370	450	686	827
					% I 24	36·4	37	59
DPN	2·9	4·0	2·2	2·4	ε	ε	ε	ε
Res. phosphorus	3·5	3·6	6·1	8·7	421	477	648	624
					% I 27	39	35	44

~ = labile phosphate bonds.

The washing and incubation were performed in a Warburg apparatus. After 5 min. gassing and 10 min. pre-incubation, a known amount of phosphorus-32 was tipped in the incubation medium and the incubation continued for another 10 min. The amount of glucose still present in the diaphragm at the moment the phosphorus-32 was added to the medium can be demonstrated (Fig. 1). This amount has been determined by a chromatographic procedure. After rapid weighing, the diaphragms were homogenized in ice-cold 10 per cent trichloracetic acid, the extract centrifuged, an aliquot taken for the estimation of inorganic phosphate and phosphocreatine and specific activities. The remaining supernatant was processed using a chromatographic procedure, essentially identical to that described in a previous report[15]: adenosine triphosphate (ATP), adenosine diphosphate(ADP), adenosine monophosphate (AMP), diphosphopyridine nucleotide (DPN), and the sum of uridine and guanosine phosphates (U + G) were thus separated, their amounts and their specific activities measured. The 'residual phosphate' comprising mainly the phosphorylated intermediates of glycolysis has also been determined. In all cases, the total phosphorus and radioactivity recovered after the fractionation procedure amounted to 90 per cent–100 per cent of the original phosphorus and radioactivity present in the trichloracetic acid extract. It should be mentioned that the amount and specific radioactivity of intracellular inorganic phosphate has been calculated from the total inorganic phosphate and the extracellular phosphate present in the incubation medium assuming an extracellular space of 22 per cent as measured by Kipnis and Cori on the cut diaphragm[2].

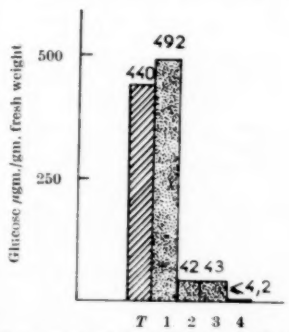


Fig. 1. Glucose content of rat diaphragm incubated under various conditions. *T*, Theoretical value for (1) (extracell. space, 22 per cent); 1, washed 5 min., 37°, Krebs–Ringer glucose, 2 mgm./ml.; 2, washed 5 min., 37°, no glucose; 3, washed 5 min., 37°, no glucose, insulin, 10 μgm./ml.; 4, same as 3 plus incubation fresh medium 15 min.

Table 1 shows a characteristic result obtained with normal rat diaphragms in absence or in presence of added insulin (10 μgm./ml.) or glucose (2 mgm./ml.).

From these results the following conclusions may be drawn: (1) There seems to be no influence of insulin or glucose on the amount of the different phosphate fractions in the rat diaphragm except so far as the 'residual phosphate' is concerned. The amount of this phosphate fraction increases in the presence of glucose and still more with glucose + insulin, as may be expected if it consists mainly of glycolytic intermediates. (2) There is no influence of insulin or glucose on the rate of penetration of radioactive phosphorus into the diaphragm of the normal rat, as visualized by the ratio of the specific activities, namely, intracellular phosphate/extracellular phosphate. (3) There is a striking enhancement of the labelling of phosphocreatine, ATP, ADP, uridine and guanosine phosphates by insulin in the presence or absence of added glucose. The increase of specific activity of these fractions with respect to intracellular phosphates averages 50 per cent for phosphocreatine (PC), 80 per cent for ATP, and 100 per cent for ADP. No significant labelling of ribose-linked phosphoryl groups such as AMP or DPN occurred within our limited times of incubation. (4) In the presence of glucose there is a definite rise of the labelling of ATP both with or without insulin. There is a slight glucose effect on the specific activities of phosphocreatine and the 'residual fraction' and a marked effect on the labelling of the uridine and guanosine phosphates. But there is no influence whatsoever of glucose on labelling of ADP, whereas insulin exerts its most striking effect on the labelling of this substance.

It is thus clear that insulin has a marked effect on the labelling of phosphorylated coenzymes even in the absence of any measurable amounts of glucose within or without the cell. The exceedingly small amount of glucose found in the diaphragm with the conditions under which the present experiments were carried out could as well have arisen from the limited breakdown of labile esters like glucose-1-phosphate or of glycogen itself. The marked effect of insulin on the labelling of the terminal phosphate group of ADP, which seems to be in equilibrium with the second phosphorylated group of ATP, is what one might expect to occur if amino-acid activation including a 'pyrophosphate split' of ATP (ref. 13) and subsequent

re-phosphorylation of AMP by an adenylate kinase system would occur. However, many other metabolic events such as, for example, activation of fatty acids[12] and increased dephosphorylation of ADP by myokinase could equally well explain the increased formation of AMP.

Finally, it must be borne in mind that although insulin does not appear to increase the penetration of radioactive phosphate into the cell of the diaphragm, a mechanism whereby insulin could promote penetration of phosphate into a restricted compartment of the muscle cell where re-phosphorylation of AMP to ADP and ATP normally occurs, thus increasing the actual labelling of the two terminal phosphorylated groups of ATP, cannot be excluded at present.

P. Volfin
D. Eboué
H. Clauser

Laboratoire de Chimie biologique
de la Faculté des Sciences de Paris,
96 Bld. Raspail,
Paris VI.

[1] Levine, R., and Goldstein, M. S., *Recent Progress in Hormone Res.*, **7**, 343 (1955).
[2] Kipnis, D. M., and Cori, C. F., *J. Biol. Chem.*, **224**, 681 (1957).
[3] Morgan, H. E., Henderson, M. J., Regen, D. M., and Park, C. R., *J. Biol. Chem.*, **236**, 253 (1961).
[4] Winegrad, A. I., and Renold, A. E., *J. Biol. Chem.*, **233**, 267. 273 (1958).
[5] Ball, E. G., Martin, D. B., and Cooper, O., *J. Biol. Chem.*, **237**, 774 (1959).
[6] Kipnis, D. M., and Noall, M. W., *Biochim. Biophys. Acta*, **28**, 226 (1958).
[7] Manchester, K. L., and Young, F. G., *Biochem. J.*, **75**, 487 (1960).
[8] Manchester, K. L., and Young, F. G., *Biochem. J.*, **77**, 386 (1960).
[9] Manchester, K. L., and Krahl, M. E., *J. Biol. Chem.*, **234**, 2938 (1959).
[10] Tepperman, J., and Tepperman, H. M., *Pharmacol. Revs.*, **12**, 301 (1960).
[11] Barrnett, R. J., and Ball, E. G., *Science*, **129**, 1282 (1959).
[12] Berg, P., *J. Biol. Chem.*, **222**, 991 (1956).
[13] Hoagland, M. B., *Biochim. Biophys. Acta*, **16**, 288 (1955).
[14] Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Tissue Metabolism*, 119 (Burgess Pub. Co., Minneapolis, 1951).
[15] Volfin, P., Ciauser, H., Gautheron, D., and Eboué, D., *Bull. Soc. Chim. Biol.*, **43**, 107 (1961).

Effects of the Sulphur Amino-Acids on the Vitamin A Content in Liver of Rats

Recently we reported[1] the effect of vitamin A on the cystine content in the skin of rats and suggested a causative relationship between vitamin A and sulphur amino-acids. The present work was designed to investigate the effects of dietary supplementation of sulphur amino-acids on the vitamin A content in liver of rats.

The test animals used were rats of both sexes of the Wistar strain weighing about 40 gm. They were housed in individual compartments with screen bottoms and fed the basal diet deficient in sulphur amino-acid content as shown in Table 1.

Table 1. Composition of the Basal Ration

	Exp. 1	Exp. 2
Milled rice	60 gm.	92 gm.
De-fatted soybean meal	26 ,,	6 ,,
Soybean oil	5 ,,	2 ,,
Dried yeast	5 ,,	0 ,,
Sodium chloride	0 ,,	1 ,,
Calcium carbonate	0 ,,	1 ,,
Salt mixture (Hegsted)	4 ,,	0 ,,
Supplements per rat daily		
Thiamine	0 μgm.	20 μgm.
Riboflavin	0 ,,	50 ,,
Vitamin A palmitate	100 ,,	1 ,,
α-Tocopherol	300 ,,	0 ,,

In the first experiment we investigated the effect of supplementary methionine, cystine, lysine and gelatin on the basal diet. Five groups of rats were given different diets as described in Table 2. Rats in group 1 were fed the basal diet and used as control. Groups 2, 3, 4 and 5 were given methionine, cystine, lysine and gelatin respectively. The amount of gelatin was so adjusted that the lysine content in it was equal to the amount of lysine used in group 4. During the four-week feeding period, the amount of diet was restricted to 6 gm. per rat daily during the first week, and thereafter increased by 1 gm. a week. At the end of four weeks the animals were killed. Pieces of liver from the median and left lateral lobes were removed immediately, weighed and saponified with alcoholic potassium hydroxide, and the amount of vitamin A extracted by di-ethyl ether was measured by the αα′-glycerol dichlorohydrin method[2]. For determination of the sulphydryl activity of the liver, another weighed piece was homogenized and incubated with monoiodoacetic acid at 37° C. for 1 hr. and the liberated iodine in its deproteinized filtrate was measured by Mori's method[3]. The absorption coefficient (E) obtained on a colorimeter was taken as the measure of sulphydryl activity.

The result of the first experiment showed that the vitamin A content in liver of animals fed the methionine-supplemented diets, but not those given the lysine- or gelatin-supplemented diets, was strikingly elevated above that of the control. Cystine supplementation showed a moderate increase in vitamin A content of liver over that of the control animals. The sulphydryl activity showed close parallelism with vitamin A content; the highest value was obtained with a supplement of methionine, the next highest being that of cystine.

In the second experiment we investigated the effect of methionine on the vitamin A content in liver of rats fed an inadequate supply of protein and vitamin A. In this case, a basal diet of lower protein content than that in the first experiment was used and was given in conjunction with the liberal or small dosage of vitamin A, the small dosage being inadequate to allow accumulation of the vitamin in the liver. Amount of supplement of the four groups and analytical data are shown in Table 3.

The results of the second experiment showed that with the addition of methionine to the diet of low protein content, the differences in the content of vitamin A in livers were less pronounced but still significant; in other words, the vitamin content was higher than those of the unsupplemented regardless of the presence of an ample or minimum supply of vitamin A.

Present findings indicate that the dietary supplement of sulphur amino-acids, especially methionine, elevated the vitamin content in the liver of rats, while lysine or gelatin increased it a little or not at all above that of rats fed the basal diet. The basal and supplemented groups of rats were normal in general appearance and no apparent difference was observed between their growth-rates. It is remarkable that the sulphur amino-acids alone had an increasing effect on the vitamin A content in liver. This is in agreement with our suggestion[1] that vitamin A is involved in the metabolism of sulphur amino-acid.

Concurrent with the increase of vitamin A content in liver, the sulphydryl activity in liver and muscle of rats fed a diet supplemented with methionine increased. In this connexion, Flesch *et al.*[4] noticed that vitamin A was able to unite with the sulphydryl

Table 2. Effect of Methionine, Cystine, Lysine and Gelatin on the Vitamin A Content in Liver of Rats
(Values shown with mean and standard deviation of five rats in each group)

Group No.		1	2	3	4	5
Per 100 gm. of diet	DL-Methionine (gm.) L-Cystine (gm.) DL-Lysine (gm.) Gelatin (gm.)		0·2	0·2	0·2	4·3
Body-weight	Initial (gm.) Gain per day (gm.)	40·4 ± 8·3 2·0 ± 0·12	40·4 ± 6·6 2·0 ± 0·11	40·2 ± 5·9 2·0 ± 0·49	40·8 ± 5·4 2·1 ± 0·12	40·8 ± 5·3 2·0 ± 0·28
Liver	Weight/Body wt. (per cent)	4·52	3·59	3·71	3·61	3·67
Liver	Vitamin A (μgm. per cent) Sulphydryl activity*	309 ± 50 100 ± 27·6	774 ± 141 152 ± 21·8	494 ± 111 128 ± 28·2	347 ± 40 125 ± 21·8	306 ± 60 103 ± 18·4

* Relative value against group 1 by reading of colorimeter.

Table 3. Effect of Methionine on the Vitamin A Content in Liver of Rats
(Values shown with mean and standard deviation of six rats in each group)

Group No.		1	2	3	4
Vitamin A per rat daily (μgm.) Methionine per 100 gm. diet (gm.)		1 0	1 0·2	100 0	100 0·2
Body-weight	Initial (gm.) Gain per day (gm.)	36·3 ± 2·1 1·86 ± 0·10	36·5 ± 2·5 1·94 ± 0·07	36·5 ± 2·5 1·96 ± 0·12	36·5 ± 3·1 1·98 ± 0·10
Liver	Vitamin A (μgm. per cent)	157 ± 52·6	231 ± 37·8	386 ± 45·0	424 ± 40·6
Muscle	Sulphydryl activity*	100 ± 16·5	146 ± 18·1	104 ± 15·7	157 ± 14·0

* Relative value against group 1 by reading of colorimeter.

groups, possibly due to the unsaturated centres, and prevented their conversion into disulphide linkages. Thus, our findings that there was a correlation between the increase of sulphydryl activity and vitamin A content are especially interesting.

Tatsuo Koyanagi
Satoshi Odagiri

Department of Agricultural Chemistry,
Iwate University,
Morioka,
Japan.

[1] Koyanagi, T., and Odagiri, S., *Nature*, **186**, 809 (1960).
[2] Sobel, A. E., and Werbin, H., *Anal. Chem.*, **19**, 107 (1947).
[3] Mori, S., *J. Agric. Chem. Soc., Japan*, **18**, 41 (1942).
[4] Flesch, P., and Goldstone, S. B., *J. Invest. Dermatol.*, **18**, 267 (1952).

Conservation of the Biological Activity of a Soluble Ribonucleic Acid

Previous investigations[1] carried out in one of our laboratories have shown that nucleic acids could be precipitated as quaternary ammonium salts, soluble in various organic solvents, and that they could be regenerated from these organic solutions as the sodium salts on addition of concentrated solutions of sodium chloride. This treatment does not affect the infectivity of tobacco mosaic virus ribonucleic acid (RNA)[2].

We thought that it might be interesting to see if such a treatment could also be applied to soluble RNA (S-RNA) and to study its influence on the ability of S-RNA to accept amino-acids activated as amino-acyl adenylates.

S-RNA was prepared from baker's yeast according to the technique of Monier *et al.*[3]. This S-RNA was precipitated by addition at 0° of trimethylhexadecyl-ammonium bromide in the proportion of one quaternary ammonium group for one nucleotidic phosphorus. The precipitate of quaternary ammonium ribonucleate, washed three times with distilled water, was soluble in absolute ethyl alcohol. On the addition of an equal volume of 3 *M* sodium chloride solution, the S-RNA was regenerated as the sodium salt, and, after three washings with a mixture of equal volumes of ethyl alcohol and 3 *M* sodium chloride, it was redissolved in distilled water.

The activity as valine-acceptor of the so-treated S-RNA was compared with that of a control S-RNA, under the following incubation conditions: 100–150 μgm. of S-RNA, 10 μmoles of adenosine triphosphate (ATP)-Mg^{++} and 0·333 μmole of ^{14}C-DL-valine (corresponding to 1 μc.) were incubated in a final volume of 1 ml. of *tris*-(hydroxymethyl)aminomethane hydrochloride buffer 0·025 *M* pH 7·6 containing 0·0025 *M* magnesium chloride, at 37° for 30 min., in the presence of a valine-activating enzymic preparation. The enzymic preparation was the *S*4 fraction of rat ascites hepatoma cells, prepared according to Hecht *et al.*[4]. The S-RNA was precipitated and isolated, and its radioactivity measured under the conditions described by these authors[4]. The results obtained are summarized in Table 1.

Table 1. ^{14}C-Valine Incorporation Rate of Control S-RNA and Treated S-RNA

	C.p.m./mgm. S-RNA	Incorporated valine (mμmoles)	
Control S-RNA	988	2·13	2·17
	1,024	2·21	
S-RNA precipitated by quaternary ammonium and regenerated	1,024	2·21	2·37
	1,167	2·52	

The biological activity of S-RNA was suppressed neither by the treatment with the quaternary ammonium salt nor by the dissolution in an organic solvent. Instead, a slight increase of the specific activity was observed, in agreement with the chromatographic behaviour of the treated S-RNA and the control S-RNA on 'Ecteola'-cellulose columns[5,6].

The elution with a 0–1 *M* sodium chloride gradient of the control S-RNA showed evidence of a fraction which was eluted by low concentrations of sodium chloride and which was composed of oligonucleotides, inactive as amino-acid acceptors. This fraction dis-

appeared after precipitation of the S-RNA as quaternary ammonium salt and regeneration. This precipitation would thus constitute a method of purification of S-RNA, which is equivalent, so far as the elimination of inactive oligonucleotides is concerned, to chromatography on 'Ecteola'-cellulose.

In summary, after treatment with a quaternary ammonium salt, S-RNA can be dissolved in an organic solvent and regenerated from this solution as the sodium salt, without loss of its biological activity.

Experiments are in progress to take advantage of this dissolution in an organic solvent and attempt a fractionation of S-RNA.

J. H. Weil
J. P. Ebel

Laboratoire de Chimie Biologique,
Faculté de Pharmacie,
Strasbourg, France.

R. Monier*

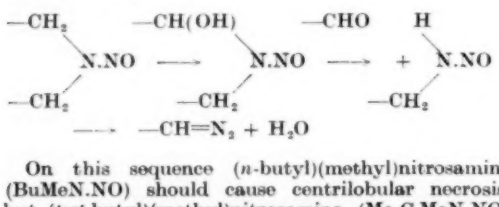
Laboratoire de Biochimie,
Fondation Curie,
Paris.

* Present address: Laboratoire de Chimie Biologique, Faculté des Sciences, Marseille.

[1] Aubel-Sadron, G., Beck, G., Ebel, J. P., and Sadron, C., *Biochim. Biophys. Acta*, **42**, 542 (1960).
[2] Hirth, L., Lebeurrier, G., Aubel-Sadron, G., Beck, G., Ebel, J. P., and Horn, P., *Nature*, **188**, 689 (1960).
[3] Monier, R., Stephenson, M. L., and Zamecnik, P. C., *Biochim. Biophys. Acta*, **43**, 1 (1960).
[4] Hecht, L. P., Zamecnik, P. C., Stephenson, M. L., and Scott, J. F., *J. Biol. Chem.*, **233**, 954 (1958).
[5] Bosch, L., Bloemendal, H., and Sluyser, M., *Biochim. Biophys. Acta*, **41**, 444 (1960).
[6] Monier, R., and Legault-Demare, J. (unpublished results).

Mechanism of the Hepatoxic Action of Dialkylnitrosamines

Dimethylnitrosamine[1] and some other dialkylnitrosamines[2,3] cause centrilobular necrosis in rats. Schoental and Rose[4] suggested independently that centrilobular necrosis was caused by diazo-alkanes formed by the metabolic oxidation of the nitrosamines in the liver *in vivo*[5,6]. The sequence of reactions is presumed to be :



On this sequence (*n*-butyl)(methyl)nitrosamine (BuMeN.NO) should cause centrilobular necrosis, but (*tert*-butyl)(methyl)nitrosamine ($Me_3C.MeN.NO$) should not. α-Oxidation of the *n*-butyl compound can give either diazomethane or diazobutane, according to which alkyl group is attacked. In the *tert*-butyl compound the *tert*-butyl group cannot be attacked in the α position, and oxidation of the methyl group leaves a compound with no α-hydrogen atom, which cannot, therefore, yield a diazo-alkane.

The two compounds were synthesized and tested on female rats (Porton Wistar strain). Both compounds were given intraperitoneally as solutions in normal saline.

The *n*-butyl compound caused acute centrilobular necrosis in doses of 100–120 mgm./kgm. in 1–2 days. Doses of 60 mgm./kgm. were without obvious effect. This compound is about one-quarter as toxic as dimethylnitrosamine, and affects the liver in a very similar way.

Twenty rats were treated with the *tert*-butyl compound. Four died from the treatment they received, so the doses given were near the maximum tolerable. Three survived 1,600 mgm./kgm. given in divided doses over 4 days, and three others survived 1,400 mgm./kgm. given over the same period. The rest survived smaller doses given over shorter periods. The survivors were killed at various times after the last dose—from the highest doses at 1, 3 or 10 days after. Various lesions were observed on histological examination, but no centrilobular necrosis in any case. Thus the *n*-butyl compound caused the typical lesion, but the *tert*-butyl compound did not, in accordance with the diazo-alkane theory.

This evidence for the diazo-alkane theory is not, of course, conclusive. It is possible that still higher doses of the *tert*-butyl compound might cause centrilobular necrosis. Alternatively, perhaps the *tert*-butyl compound does not reach the sites in the liver at which metabolism takes place. This last possibility is being investigated with nitrosamines labelled with carbon-14.

I thank Dr. P. N. Magee for carrying out the histological examinations, Dr. A. R. Mattocks for preparing the specimens of nitrosamines, and Mr. J. Rose for technical assistance.

D. F. Heath

Toxicology Research Unit,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton, Surrey.

[1] Barnes, J. M., and Magee, P. N., *Brit. J. Indust. Med.*, **11**, 167 (1954).
[2] Schmähl, D., Preussmann, R., and Hamperl, H., *Naturwiss.*, **47**, 89 (1960).
[3] Magee, P. N. (personal communication).
[4] Rose, R. F., *The Evaluation of Drug Toxicity*, 116 (Churchill, London, 1958).
[5] Dutton, A. H., and Heath, D. F., *Nature*, **178**, 644 (1956).
[6] Magee, P. N., *Biochem. J.*, **64**, 676 (1956).

PHYSIOLOGY

Cytotoxic Effect of Aminoacetonitrile on Fibroblast Cultures and its Prevention by Histidine

The understanding of the ætiological factors involved in the curious poisoning arising from the ingestion of *Lathyrus odoratus* seeds has been greatly advanced by the identification of β-amino-propionitrile as the active moiety of the toxic principle and the recognition of the even more potent lathyrogenic property of aminoacetonitrile (AAN)[1,2]. However, the site and nature of the primary metabolic disturbance responsible for the characteristic mesenchymal lesions produced by aminonitriles have not yet been elucidated. The present investigation was designed primarily to explore the possibility of using tissue cultures as a prospective tool for studying under *in vitro* conditions the biochemical mechanism underlying the toxic action of AAN.

The experiments were performed regularly with chick embryo fibroblasts grown in a modified *M*–150 medium of Morgan *et al.*[3] consisting of Hanks's salt solution, a mixture of amino-acids and vitamins and fortified with 2 per cent calf serum. In most experiments 3–4 days-old cultures were used and AAN was added with fresh medium following the removal of the initial culture fluid. Under such conditions

Table 1. PREVENTION OF CYTOTOXIC ACTION OF AMINOACETONITRILE BY HISTIDINE

Aminoacetonitrile (μmoles per ml.)	L-Histidine (μmoles per ml.)	Time course of cytotoxic effect (hr.) 24	48	72	96	120
0	0	—	—	—	—	—
0	0·25–4	—	—	–	—	—
0·7	0	+	+ +	+ + + +	+ + + +	+ + + +
0·7	0·25	—	—	+ + +	+ + + +	+ + + +
0·7	0·50	—	—	+ +	+ + +	+ + + +
0·7	1·00	—	—	—	—	+ +
0·7	2·00	—	—	—	—	—
1·0	0	+ + + +	+ + + +	+ + + +		
1·0	1·00	—	+ +	+ + + +		
1·0	2·00	—	—	+		
3·0	0	+ + + +	+ + + +	+ + + +		
3·0	1·0	+ +	+ + + +	+ + + +		
3·0	2·0	+ +	+ +	+ + +		
3·0	4·0	+	+ +	+ +		

Suitable dilutions of aminoacetonitrile hydrosulphate and histidine hydrochloride neutralized with NaOH were added to 3-day-old cultures along with the replacement medium *M*-150 (ref. 3). Incubation (at 37° C.) was continued and the cultures were examined microscopically at the intervals indicated. All experiments were performed in 5-replicate tubes.

Key: — No effect; +, scattered appearance of rounded cells but cell-sheet intact; + +, incipient loosening of cell texture; + + +, pronounced fragmentation of cell sheet, large number of loose rounded cells; + + + +, disintegration of cell sheet complete, rounded cells and cell debris.

AAN (100 μgm. per ml.) produced a series of progressive alterations manifested by swelling of the nuclei, rounding of the cells and enlargement of their volume until the fibroblastic morphology was no longer recognizable: concurrently, fragmentation of the cell-sheet became increasingly pronounced and finally complete disintegration of the culture took place at the end of 2–3 days after the addition of AAN. With higher levels of AAN (150–400 μgm. per ml. medium) the course of the toxic phenomenon was considerably accelerated and short-circuited.

The noxious effect of AAN was most prominent in young, actively proliferating fibroblasts and appeared to decrease with increasing age of the culture. This observation may be correlated with the striking enhancement of AAN toxicity occurring in fibroblast cultures supplemented with microgram quantities of triiodothyronine; triiodothyronine has been shown previously to stimulate considerably the metabolic rate of chick fibroblasts[4]. Both findings seem to suggest that high metabolic activity is essential for poisoning to occur. It may be also relevant to mention in this connexion that the susceptibility to experimental lathyrism is highest in weanling rats and declines sharply with increasing age of the animals[5].

Mouse fibroblasts exhibited an essentially similar response to AAN although their sensitivity was of a somewhat lower order than that of the chicken fibroblasts. On the other hand, two epithelial lines tested (kindly supplied by Dr. J. F. Morgan), namely, human kidney cells and the *FL* strain of human amnion cells, proved to be refractory to AAN even at doses threefold higher than those lethal for chick fibroblasts.

Of particular interest was the observation that addition of an enzymic hydrolysate of lactalbumin to the culture medium (10–20 mgm. per ml.) resulted in a significant delay or even complete prevention of the toxic phenomenon induced by AAN under the usual test conditions. The latter finding, which appears to bear directly on the mode of action of AAN, may be related to the repeated observations that the course and severity of the *Lathyrus* poisoning in rats can be modified considerably or attenuated by maintaining the animals on protein-rich diets[6,7].

The protective effect of lactalbumin could be duplicated by raising the amino-acid concentration in the culture medium. Furthermore, out of 20 natural amino-acids tested singly or in various combinations, only histidine overcame the cytopathic action of AAN. As shown in Table 1, L-histidine at 2×10^{-3} *M* gave complete protection against 7×10^{-4} *M* AAN. When tested against graded amounts of AAN, the effective dose of histidine varied in a roughly parallel manner with the changing levels of AAN.

Further work revealed that D-histidine is equally effective as its natural (L-) enantiomorph in counteracting the toxicity of AAN. Histamine and imidazole, on the other hand, failed to exhibit a protective effect.

It may be concluded, therefore, that the ability of histidine to prevent the cytotoxic action of AAN is probably unrelated to its role as an essential amino-acid since the latter property is known to be stereospecific with respect to the L-configuration[8]. The possible relationship of the protective property of histidine to its well-known metal-binding capacity[9] is being examined.

Addendum. Recent experiments revealed that the cytotoxic action of AAN on fibroblasts requires the presence of traces of copper ions in the culture medium. In the light of the latter finding, the protective effect of histidine appears to be attributable to its copper-chelating property.

We acknowledge the assistance of Miss M. Faierman.

J. MAGER
Z. FUCHS
S. HALEVY
I. SCIAKY

Department of Biochemistry,
The Alpha Omega Research Center,
The Hebrew University–Hadassah
Medical School,
Jerusalem, Israel.

[1] Schilling, E. D., and Strong, F. M., *J. Amer. Chem. Soc.*, **77**, 2843 (1955).
[2] Stamler, F. W., *Proc. Soc. Exp. Biol. Med.*, **90**, 294 (1955).
[3] Morgan, J. F., Campbell, M. E., and Morton, H. J., *J. Nat. Cancer Inst.*, **16**, 557 (1955).
[4] Halevy, S., and Avivi, L., *Exp. Cell Res.*, **20**, 458 (1960).
[5] Selye, H., *Rev. Canad. Biol.*, **16**, 1 (1957).
[6] Dasler, W., *Proc. Soc. Exp. Biol. and Med.*, **85**, 485 (1954).
[7] Gardner, A. F., *Amer. J. Clin. Nutr.*, **7**, 213 (1959).
[8] Hearon, J. Z., *J. Nat. Cancer Inst.*, **9**, 1 (1948).
[9] Eagle, H., *J. Biol. Chem.*, **214**, 839 (1955).

Incorporation of ^{14}C-glycine by Sub-Nuclear Preparations from Rat Liver Nuclei

IT has been shown previously[1] that rat liver nuclei isolated in 0·25 *M* sucrose solution will incorporate ^{14}C-glycine into protein *in vitro*. The possibility of non-specific exchange of glycine was eliminated[2]. It was shown that radioactivity was retained after re-incubation of labelled nuclei for 2 hr. in a 200-fold excess of unlabelled glycine. The incorporation of glycine by nuclear preparations was inhibited by 2,4-dinitrophenol (DNP), azide, and anoxia.

The ability to incorporate glycine was retained after the nuclear preparations had been subjected to various treatments. Suspending the nuclei in water, freezing and thawing this preparation up to four times, or even freeze-drying and resuspending the nuclei in water did not reduce the ability to incorporate by more than 30 per cent. Treatment with a detergent, 'Triton *X* 100', was no more effective. Microscopic examination showed these treated nuclei to be structurally intact.

In a further attempt to disrupt the nuclei, the preparation was subjected to sonic disintegration. Microscopic examination showed that complete disintegration was obtained after 15 min. at 20,000 oscillations per sec. using a Mullard ultra-sonic disintegrator. This sonicate incorporated ^{14}C-glycine without any loss of activity compared with the intact nuclei (Table 1).

Table 1

Preparation	Counts/min. at infinite thickness
Intact nuclei	192
Sonically disintegrated nuclei	204
Supernatant after $6{\cdot}3 \times 10^6$ $g_{av.}$ min.*	171
Supernatant after $4{\cdot}41 \times 10^7$ $g_{av.}$ min.	156
Residue after $4{\cdot}41 \times 10^7$ $g_{av.}$ min.	380

For incorporation of 2-^{14}C-glycine into nuclear protein the system used was: sodium phosphate buffer, *p*H 7·4, 20 m*M*; NaCl, 20 m*M*; ^{14}C-glycine, 1 μc.; 1·5 ml. of a sub-nuclear suspension in 0·25 *M* sucrose; water to 2·5 ml.; temperature, 38° C.; for determination of radioactivity, see Rees and Rowland (ref. 1).

* Centrifugal force developed at the mid-point of the tube.

The sonicate was centrifuged at 105,000*g* for 60 min. in a 'Spinco' model *L* ultra-centrifuge at 0° C. Using the same incubation medium as for intact nuclei, the supernatant was able to incorporate ^{14}C-glycine and this incorporation was not inhibited by DNP or anoxia. Centrifugation of this supernatant for 7 hr. at 105,000*g* yielded a pellet which on re-suspension in 0·25 *M* sucrose very actively incorporated ^{14}C-glycine. The supernatant also retained ability to incorporate (Table 1).

Sub-nuclear particles have been prepared from calf-thymus nuclei by extraction with *tris* buffer (*p*H 7·1)[3]. These particles incorporate amino-acids but, in contrast to the preparations described in this paper, they require a system containing the nuclear *p*H 5 enzymes[3], adenosine triphosphate, guanosine triphosphate and a source of generating adenosine triphosphate.

This work was financed by a grant from the British Empire Cancer Campaign.

K. R. Rees
Hazel F. Ross
G. F. Rowland

Department of Chemical Pathology,
University College Hospital
Medical School,
London, W.C.1.

[1] Rees, K. R., and Rowland, G. F., *Biochem. J.*, **78**, 89 (1961).
[2] Allfrey, V. G., Mirsky, A. E., and Osawa, S., *J. Gen. Physiol.*, **40**, 451 (1957).
[3] Frenster, J. H., Allfrey, V. G., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **46**, 432 (1960).

Fate of Tritiated Noradrenaline at the Sympathetic Nerve-Endings

Previous work in this Laboratory has shown that tritiated adrenaline and tritiated noradrenaline are taken up and retained in certain tissues for long periods of time[1,2]. It was also demonstrated that chronic denervation of sympathetic nerves resulted in the decreased ability of denervated tissues to take up administered tritiated noradrenaline and adrenaline[2]. These observations indicated that sympathetic nerve-endings selectively took up and retained circulating catecholamines. This property enabled us to label the nerve-endings with tritiated noradrenaline and to study its fate on liberation from sympathetic nerves.

In the experiments described here cats were given tritiated noradrenaline, the splenic nerve stimulated in the manner described by Brown and Gillespie[4], and the tritiated noradrenaline and its metabolites measured in the venous outflow. Because of the relatively large amount of tritiated noradrenaline present in the blood supplying and leaving the spleen, this organ was isolated and perfused with blood from a donor cat that was free of radioactive material. Cats were given 200 μc./kgm. DL-^{3}H-noradrenaline (20 mc./mgm.) in the forelimb vein. Three hours later the animals were anæsthetized with pentobarbitone and given 3000 U.S.P. units of heparin. The small and large intestines were tied off and removed. The blood vessels between the spleen, stomach and omentum were tied and cut. Polyethylene tubing ('*PE* 200' Clay-Adams) was inserted into the superior mesenteric vein and the tubing clamped. The superior mesenteric artery was tied off close to the aorta and cut, and the splenic artery was separated from the connecting tissues. Polyethylene tubing ('*PE* 20') filled with heparinized saline was inserted into the artery branching from the splenic artery to the stomach and was used to inject drugs into the spleen. A second cat received pentobarbitone and heparin, and its femoral artery cannulated with polyethylene tubing ('*PE* 200') and connected to the splenic artery of the first cat. The blood supplied from the donor cat was replaced by blood obtained from a third cat. The portal vein of the first cat was tied and cut, and the tubing in the mesenteric vein opened. After the remaining connexions were tied and cut, the spleen was placed in a saline water bath at 37° C. The splenic nerve was placed on a dipolar platinum electrode and was coated with paraffin. The splenic nerve was stimulated with 10 V. for a duration of 1 msec. a stimulus and a frequency of 10 or 30/sec. An equal number of

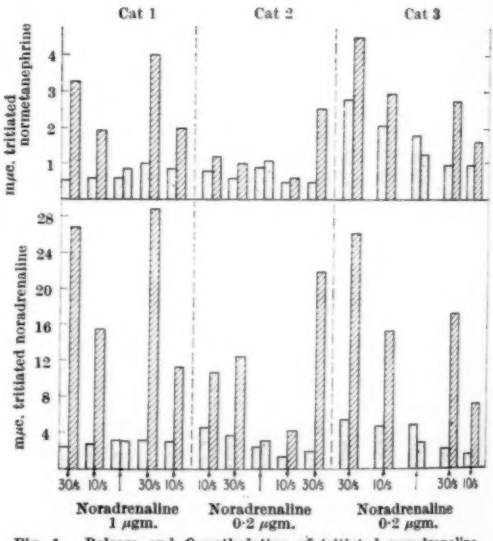


Fig. 1. Release and *O*-methylation of tritiated noradrenaline after stimulation of the splenic nerve. Cats were given tritiated noradrenaline intravenously and the spleen was prepared as described in the text. Splenic nerves were stimulated at a frequency of 10 or 30/sec. Cats 1 and 3 received a total of 300 stimuli for each period and cat 2 received 200 stimuli. Blood was collected 2·5 min. before and 2·5 min. during and after stimulation from cats 1 and 3 and 2·0 min. for each period from cat 2. Non-radioactive noradrenaline was injected into the splenic artery. Each bar represents tritiated noradrenaline or tritiated normetanephrine in mμc. found in each period. Stippled bars are tritiated noradrenaline and tritiated normetanephrine measured before stimulation, and striped bars are tritiated noradrenaline and tritiated normetanephrine during and after stimulation

stimuli was given in all cases. A Grass stimulator, model *S 4B*, was used. Blood was collected for 2·0 or 2·5 min. before and 2·0 or 2·5 min. during and after stimulation. There was a 7-min. interval between each group of stimuli. Blood was collected in graduated centrifuge tubes containing two drops of heparin solution and the hæmatocrit was determined. An aliquot of plasma was deproteinized with 10 ml. of 0·4 *N* perchloric acid and tritiated noradrenaline was determined as described in refs. 1, 2. Tritiated normetanephrine was estimated on the effluent from the alumina column by adsorption on 'Dowex 50' (ref. 5). Results are expressed as mμc. tritiated noradrenaline released or tritiated normetanephrine formed in each period.

After each stimulation there was a marked increase in the concentration of tritiated noradrenaline in the venous outflow (Fig. 1). A greater amount of the tritiated catecholamine was released after 30 stimuli/sec. than after ten stimuli. During each stimulation period there was a contraction of the spleen. The release of tritiated noradrenaline, however, was unrelated to this contraction since a similar contraction induced by the administration of non-radioactive noradrenaline directly into the splenic artery did not increase the outflow of tritiated noradrenaline (Fig. 1). There was a small amount of tritiated noradrenaline released spontaneously without stimulation.

After each stimulation there was a concomitant increase in tritiated normetanephrine (Fig. 1). The amount of tritiated normetanephrine found was directly related to the amount of tritiated noradrenaline released. The de-aminated metabolites (3,4-dihydroxyphenylglycol and 3,4-dihydroxymandelic acid) in the venous outflow were also determined[5]. The amount of these compounds found were small (0·2 mμc./period) and were not elevated during stimulation.

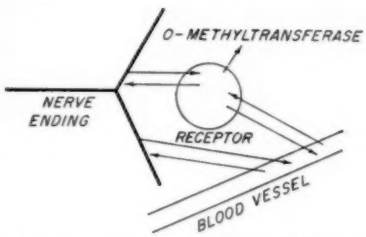


Fig. 2. Fate of noradrenaline at the sympathetic nerve ending

Our earlier work has shown that sympathetic nerve endings or neighbouring structures take up and bind circulating catecholamines[1–3]. In this communication we have demonstrated that stimulation of these nerves releases tritiated noradrenaline which was taken up previously from the circulation. This indicates that the neurohumour, noradrenaline, can be taken up from the blood-stream, bound and then released on stimulation. The *O*-methylated metabolite tritiated normetanephrine was also elevated after each series of stimulations.

It can be concluded that the noradrenaline liberated from the nerve endings reacts with the receptor, and a part is discharged into the bloodstream, a part *O*-methylated by catechol-*O*-methyl transferase[6] and a part bound again at the sympathetic nerve endings. The fate of noradrenaline at the sympathetic nerve ending is shown schematically (Fig. 2).

We wish to thank Mr. R. W. Patrick for his competent technical help.

G. Hertting
J. Axelrod

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland.

[1] Axelrod, J., Weil-Malherbe, H., and Tomchick, R., *J. Pharmacol. and Exp. Therap.*, **127**, 251 (1959).
[2] Whitby, L. G., Axelrod, J., and Weil-Malherbe, H., *J. Pharmacol. and Exp. Therap.*, **132**, 193 (1961).
[3] Hertting, G., Axelrod, J., Kopin, I. J., and Whitby, L. G., *Nature*, **189**, 66 (1961).
[4] Brown, G. L., and Gillespie, J. S., *J. Physiol.*, **138**, 81 (1957).
[5] Kopin, I. J., Axelrod, J., and Gordon, E., *J. Biol. Chem.* (in the press).
[6] Axelrod, J., *Science*, **126**, 400 (1957).

Phenylalanine as Inhibitor of Transport of Amino-Acids in Brain

In phenylketonuria the tissues are unable to metabolize phenylalanine to tyrosine, resulting in abnormally high levels of phenylalanine in body fluids, together with a number of disorders of tyrosine metabolism[1,2].

It has been shown that certain amino-acids can interfere with the transport of other amino-acids in tissues[3,4]. Thus it is possible that the high levels of phenylalanine in phenylketonuria might produce a similar type of interference and result in abnormal metabolism of free amino-acids generally.

To determine whether such an interference might occur, the effect of phenylalanine on the uptake of five amino-acids by brain tissue has been investigated, using an *in vitro* technique described elsewhere[5]. Slices of rat brain were agitated for 1 hr. at 37° C. in Krebs's bicarbonate saline which contained the amino-acids under investigation. The concentration of the particular amino-acid being investigated was then estimated in tissue and suspending medium by a colorimetric method[6–8]. There was no evidence that phenylalanine when present in equimolar amounts interfered with the estimation of any of the other amino-acids; at a concentration ten-times higher it interfered only with the estimation of arginine, but to a negligible extent. Recoveries of appropriate amounts of tyrosine added to tissue initially agitated for 1 hr. in medium free of amino-acid were found to be low (87–88 per cent); values found for tyrosine in tissue have therefore been corrected by the corresponding factor. Recoveries of the other amino-acids were satisfactory. The *p*H of the suspending medium (critical in this type of experiment[5]) was measured at the end of all experiments and lay within the range 7·3–7·4. The mean blank value in tissue fluid of each amino-acid being estimated was not greater than 0·35 m*M*.

Results have been expressed in the form of the 'concentration difference' (concentration of amino-acid in tissue fluid minus concentration of amino-acid in suspending medium), which is an indication of the net effectiveness of the transport mechanism for the particular amino-acid being investigated.

Table 1 shows that phenylalanine not only interferes with transport into brain tissue of all the amino-acids investigated, but also that it inhibits uptake of tyrosine considerably more than that of any of the other four amino-acids. It follows that the high phenylalanine-level of phenylketonuria probably causes a similar interference with movements of tyrosine and, to a smaller extent, of other amino-

Table 1. Effect of Phenylalanine on Transport of Amino-acids

Amino-acid	Concentration difference developed between slices and suspending medium (m*M*)		Inhibition by L-phenylalanine (per cent)
	When present alone	In the presence of an equimolar amount of L-phenylalanine	
L-Proline	8·48 ± 0·73 (16)	7·71 ± 0·67 (16)	9
L-Histidine	15·6 ± 1·1 (12)	9·0 ± 1·6 (20)	42
L-Arginine	2·70 ± 0·56 (16)	1·47 ± 0·32 (15)	46
L-Ornithine	3·44 ± 0·31 (12)	1·82 ± 0·14 (12)	47
L-Tyrosine	3·88 ± 0·32 (13)	1·15 ± 0·10 (12)	70

Initial concentration of each amino-acid in suspending medium 2 m*M*. Figures shown for concentration difference are mean and standard deviation with the number of samples in parentheses. Experimental period, 1 hr. 37° C.

acids in brain and other tissues of the body. The considerable interference with tyrosine transport would be of greater significance in those tissues such as brain and intestinal mucosa which transport tyrosine the most actively[9]. With tissues in which there does not appear to be active transport of tyrosine[9], movement of tyrosine would probably not be affected, as has already been shown with liver[10].

I wish to thank Prof. N. L. Edson for giving me facilities in the Department of Biochemistry, and the Medical Research Council of New Zealand for financial support.

K. D. Neame*

Department of Medicine,
Medical School,
Great King Street,
Dunedin, New Zealand.

* Present address: Department of Physiology, University of Liverpool, England.

[1] Knox, W. E., and Hsia, D. Y.-Y., *Amer. J. Med.*, **22**, 687 (1957).
[2] Meister, A., *Pediatrics*, **21**, 1021 (1958).
[3] Christensen, H. N., Riggs, T. R., Fischer, H., and Palatine, I. M., *J. Biol. Chem.*, **198**, 1 (1952).
[4] Wiseman, G. and Ghadially, F. N., *Brit. J. Cancer*, **9**, 480 (1955).
[5] Neame, K. D., *J. Neurochem.*, **6**, 358 (1961).
[6] Chinard, F. P., *J. Biol. Chem.*, **199**, 91 (1952).
[7] Macpherson, H. T., *Biochem. J.*, **40**, 470 (1946).
[8] Ceriotti, G., and Spandrio, L., *Biochem. J.*, **66**, 607 (1957).
[9] Neame, K. D., *Proc. Univ. Otago Med. Sch.*, **39**, 11 (1961).
[10] Bickis, I. J., Kennedy, J. P., and Quastel, J. H., *Nature*, **179**, 1124 (1957).

Stability of Dextran during Prolonged Storage

So far as we are aware, no measurements relating to the stability of clinical dextran solutions kept for several years have been published. Two dextran solutions made in the United States, *A* and *B*, and two made in Great Britain, *C* and *D*, were examined in 1954, and again after storage for 5 yr. at 4° C. Where applicable the methods of testing described in the British Pharmacopœia[1] were used. The optical rotation of each dextran was determined and used in calculating the concentrations of solutions for the viscosity measurements (Table 1). Dextran *D* had undergone the most change; the changes in the others were relatively small.

The renal excretion of these dextrans was measured in rabbits (Table 2). The greater part of the dextran was excreted on the first day, less than 3 per cent on the second and less than 1 per cent on the third day. Two, three or four rabbits were used for each determination and the daily results averaged; Table 2 records the totals for 3 days after injection. The results for dextran *A* suggest a change in molecular composition, but no corroboration of this was found in the other measurements.

Table 1

Dextran	Optical rotation (*a*)	Intrinsic viscosity at 25° C.	
		1954	1959
A	195·1	0·249	0·248
B	197·7	0·254	0·251
C	201·2	0·394	0·388
D	198·1	0·362	0·326

Table 2

Dextran	Percentages of injected dose excreted	
	1954	1959
A	37·2	47·6
B	43·1	39·1
C	13·5	14·6
D	15·9	15·4

The retention of each dextran in the plasma was determined by taking the average of daily estimations in the same groups of rabbits (Table 3). During five years of storage little if any change had occurred in the dextrans affecting their retention in the circulation.

Table 3. Plasma Concentration of Dextran as Percentage of Concentration 10 min. after Injection

Dextran		10 min.	Day 1	2	3	4	5	6	7
A	1954	100	28·8	11·7	0·4	0·0	0·0	0'0	0·0
	1959	100	29·6	15·2	2·25	0·8	0·0	0·0	0·0
B	1954	100	29·2	13·8	3·6	0·0	0·0	0·0	0·0
	1959	100	30·0	14·1	2·0	0·6	—	—	0·0
C	1954	100	56·8	34·8	12·3	3·8	0·7	—	0·0
	1959	100	61·0	40·9	18·3	10·0	—	—	0·0
D	1954	100	60·5	40·2	22·8	12·8	5·1	—	0·8
	1959	100	56·5	38·7	22·0	—	—	3·3	1·5

The difference in molecular composition of American and British dextrans is well illustrated by these results. The lower average molecular weight of the American dextrans is associated with shorter retention in the circulation and greater renal excretion. The British dextran, of higher average molecular weight, would induce greater aggregation of red cells *in vitro*[2] and possibly *in vivo*, too.

From our observations we conclude that during the 5-yr. period there was little, if any, change in the molecular composition of these dextran solutions and none that would be noticeable in clinical use. It is probable that any limitation of the storage 'life' of dextran solution will be imposed by defects in the container and its closure rather than by any instability of dextran solution itself.

W. d'A. Maycock

Lister Institute, Elstree.

C. R. Ricketts

Medical Research Council
Industrial Injuries
and Burns Research Unit,
Accident Hospital,
Birmingham.

[1] *British Pharmacopœia* (1958).
[2] Hardwicke, J., Ricketts, C. R., and Squire, J. A., *Nature*, **166**, 988 (1950).

PHARMACOLOGY

Effects of 7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide on Mitochondria from Rat Liver and Brain

Although little is known about the relations between *in vivo* drug action and the effects of a drug on metabolic processes *in vitro*, it has been confirmed that psychopharmacological materials in many cases act on oxidative reactions in the mitochondrial respiratory chain. The two different drugs, amytal and chlorpromazine, both, for example, have inhibi-

Table 1. Effect of CMPB on Uptake of Oxygen, Respiratory Control and Phosphorus/Oxygen Ratio of Liver and Brain Mitochondria

The phosphorylation medium consisted of phosphate-buffer, *p*H 7·4, 20 m*M*; *tris*-buffer, *p*H 7·4, 20 m*M*; adenosine diphosphate, 2 m*M*; substrate, 10 m*M*, except β-hydroxybutyrate, 30 m*M*, and malate together with pyruvate, 1 m*M*; glucose, 28 m*M*, and hexokinase in excess. Each tube contained mitochondria from 100 mgm. (glutamate), 135 mgm. or 200 mgm. (brain) wet-weight tissue, and sucrose, 50 m*M*. In the case of respiratory control adenosine diphosphate and hexokinase were omitted. The final volume was 1 ml. The vessels were incubated for 15 min. at 30°

Substrate	m*M* CMPB	Liver			Brain		
		Uptake of oxygen (μatoms)	Respiratory control (μatoms)	P/O	Uptake of oxygen (μatoms)	Respiratory control (μatoms)	P/O
Glutamate	0	2·3	0·1	2·2			
Glutamate	1	1·3	0	2·2			
Malate	0	3·6	1·4	2·1			
Malate	1	2·5	1·0	2·0			
β-Hydroxybutyrate	0	2·1	1·6	1·7			
β-Hydroxybutyrate	1	1·6	1·3	1·5			
Succinate	0	4·9	2·7	2·0	3·2	1·9	1·0
Succinate	0·4	4·6	3·0	1·8	3·4	2·6	0·8
Succinate	2·0	4·7	3·2	1·7	3·3	3·3	0·5
Pyruvate + malate	0	2·9	0·7	2·6	1·6	0·4	1·9
Pyruvate + malate	0·4	2·5	1·0	2·5	1·7	0·3	1·8
Pyruvate + malate	2·0	1·4	0·8	2·5	1·1	0·6	1·4

tory effects on the flavoprotein in the respiratory chain[1,2]. Therefore it was of interest to test whether the new drug, 7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide (CMPB, used as hydrochloride)[1] acts on mitochondrial reactions in a similar manner.

CMPB, a drug of a new chemical group, has been tested in concentrations up to 1 m*M*. With higher concentrations a precipitate is formed at the physiological *p*H 7·4. Rat liver mitochondria were prepared according to the method described by Ernster and Löw[3]. Rat brain mitochondria were isolated in 0·25 *M* sucrose containing 1 m*M* ethylenediaminetetraacetic acid (EDTA)[2] between 1,400 and 12,000*g*.

As shown in Fig. 1, CMPB is a potent swelling agent on liver mitochondria. The swelling effect, however, could be prevented completely by 1 m*M* EDTA (Fig. 1*b*), which is in accordance with earlier findings on a variety of other swelling agents[4]. Lehninger[5] has shown that mitochondrial swelling produced by various agents in a buffered saline medium, but not in 0·3 *M* sucrose, could be reversed by adenosine triphosphate and magnesium. In Fig. 1*C* it can be seen that in a potassium chloride medium, swelling induced by CMPB could be reversed partially by adenosine triphosphate alone and more effectively by adenosine triphosphate with magnesium. Using brain mitochondria, no swelling could be obtained even with higher concentrations of CMPB (2 m*M*); however, 20 m*M* phosphate, too, which causes a rapid swelling of liver mitochondria, had no effect on brain mitochondria. A resistance of brain mitochondria against swelling agents has been observed recently by Spirtes[6].

The effect of CMPB on mitochondrial oxidation and phosphorylation and the respiratory control is shown in Table 1. There is a distinct inhibition of the oxidation-rates with substrates linked with diphosphopyridine nucleotide (DPN); however, no inhibition is found using succinate as substrate. Whereas the phosphorylation with the DPN-linked substrates is almost unaffected, with succinate there is a slight but obvious depression of the ratios of phosphorus to oxygen. Similar results are obtained concerning the respiratory control. Only with succinate a marked loss of the respiratory control could be detected. With brain mitochondria analogous results were obtained.

To find out the site of action of CMPB, the 2,4-dinitrophenol (DNP)-induced adenosine triphosphatases of both types of mitochondria were examined, because it has been suggested, from the DNP-induced adenosine triphosphatase, to occur only at the first phosphorylation step at the flavin region[7]. As shown in Table 2, the DNP-induced adenosine triphosphate is markedly depressed by CMPB. This effect is even higher with brain mitochondria. Amytal, which is known to have an inhibitory effect on the flavoprotein of the respiratory chain[1], stimulates the effect of CMPB.

From these observations it is suggested that CMPB has two points of action at the respiratory chain of mitochondria. It seems to inhibit the redox reactions of the flavoprotein. However, the reactions which transfer the high-energy phosphate at the flavoprotein level probably are not influenced by this drug, because of the small uncoupling effect on the phosphorylation with DPN-linked substrates compared with succinate. Löw[7] has proposed a scheme of the DNP-induced adeno-

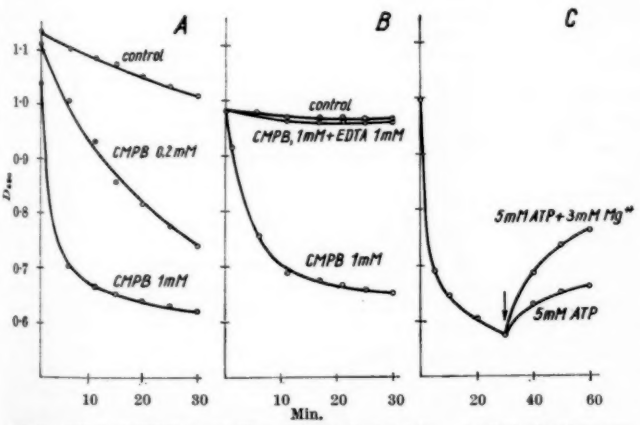


Fig. 1. Effect of CMPB on swelling of liver mitochondria. *A*, effect of various concentrations; *B*, prevention of swelling by EDTA; *C*, reversal of swelling by adenosine triphosphate and magnesium. The swelling medium consisted of *tris*-buffer *p*H 7·4, 0·02 *M* in 0·3 *M* sucrose (*A* and *B*), or in 0·125 *M* potassium chloride (*C*). Mitochondrial swelling was carried out in 1-cm. tubes at 520 mμ at room temperature

Table 2. Effect of CMPB on the DNP-induced Adenosine Triphosphatase

Each tube contained mitochondria from 100 mgm (liver), or 200 mgm. (brain) wet-weight tissue; adenosine triphosphate, 5 m*M*; *tris*-buffer, *p*H 7·4, 5 m*M*; DNP, 0·1 m*M* in 1 ml. 0·25 *M* sucrose, and was incubated for 20 min. at 30°

Additions	m*M* CMPB	*μ*moles inorganic phosphate liberated	
		Liver	Brain
	0	2·05	1·59
	0·1	1·95	1·34
	0·5	1·76	1·22
	1·0	1·53	0·89
Amytal, 10^{-3} *M*	0	1·92	1·43
Amytal, 10^{-3} *M*	0·1	1·65	1·35
Amytal, 10^{-3} *M*	0·5	1·34	1·14
Amytal, 10^{-3} *M*	1·0	1·19	0·76

sine triphosphatase reaction. In agreement with that scheme it may be suggested that the reaction between the flavoprotein and the reduced diphosphopyridine nucleotide may be the one inhibited by CMPB. The second point of action of this substance seems to be the phosphorylation steps between cytochrome *b* and the cytochrome oxidase, which is suggested from the uncoupling effect on succinate-linked phosphorylation.

No marked difference between the effects on mitochondrial reactions from brain and liver has been found. This seems to indicate that the effects shown here may have a common meaning as applied to mitochondria from different origins. Although we are not able to explain the special psychopharmacological action of this drug *in vivo* by the results obtained *in vitro*, it is of interest to note that chlorpromazine, a chemically completely different compound, which has similar pharmacological effects, acts on mitochondria in a surprisingly analogous way[8–10].

CMPB, available under the trade name 'Librium(R)', was a gift from the Deutsche Hoffmann-La Roche AG.

We thank Mrs. Brigitte Kühn for skilful technical assistance.

Bernhard Kadenbach
Walter Lührs

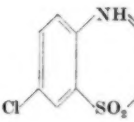
Department of Experimental and
Clinical Chemotherapy,
Robert-Rössle-Clinic,
Institute for Medicine and Biology,
German Academy of Sciences,
Berlin–Buch.

[1] Ernster, L., Jalling, O., Löw, H., and Lindberg, O., *Exp. Cell Res. Supp.*, **3**, 124 (1955)
[2] Dawkins, M. J. R., Judah, J. D., and Rees, K. R., *Nature*, **182**, 875 (1958).
[3] Ernster, L., and Löw, H., *Exp. Cell Res., Supp.*, **3**, 133 (1955).
[4] Hunter, F. E., Levy, J. F., Fink, J., Schutz, B., Guerra, F., and Hurwitz, A., *J. Biol. Chem.*, **234**, 2176 (1959).
[5] Lehninger, A. L., *J. Biol. Chem.*, **234**, 2465 (1959).
[6] Spirtes, M. A., *Biochem. Biophys. Res. Comm.*, **4**, 71 (1961).
[7] Löw, H., *Biochim. Biophys. Acta*, **32**, 1 (1959).
[8] Dawkins, M. J. R., Judah, J. D., and Rees, K. R., *Biochem. J.*, **72**, 204 (1959).
[9] Dawkins, M. J. R., Judah, J. D., and Rees, K. R., *Biochem. J.*, **73**, 16 (1959).
[10] Löw, H., *Biochim. Biophys. Acta*, **32**, 11 (1959).

A Non-diuretic Benzothiadiazine with Anti-hypertensive Properties

In recent years the benzothiadiazine diuretics have become the drugs of choice for treating mild to moderate hypertension. Although their anti-hypertensive effect is relatively weak, they are quite non-toxic and are well absorbed orally. The mechanisms by which these agents reduce blood pressure in hypertensive individuals are not known. Some investigators[1,2] have implicated natriuresis as the primary event, whereas others[3] have suggested that the concentration of circulating pressor agents is reduced. That benzothiadiazine diuretics do antagonize *in vitro* intestinal and aortic contractions has already been demonstrated[4,5]. Consequently, the possibility exists that these agents directly affect the responsiveness of vascular smooth muscle. It was, therefore, considered of interest to try to dissociate the anti-hypertensive and diuretic actions of these compounds and, further, to intensify the former action while minimizing the latter.

Accordingly, several types of benzothiadiazine analogues were synthesized by Dr. J. G. Topliss, M. H. Sherlock and Dr. N. Sperber of the Chemical Research Division, Schering Corporation, and afterwards evaluated for anti-hypertensive and diuretic activities. It soon became apparent that certain structural modifications of the benzothiadiazine nucleus produced a class of compounds that lowered arterial blood pressure acutely in laboratory animals. The most critical structural change involved the elimination of the sulphamyl group from the benzenoid moiety. A representative member of this des-sulphamyl benzothiadiazine series which has received considerable attention in our laboratories is 3-methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide (*SRG* 95213). This agent has virtually no natriuretic or diuretic activity. Indeed, it caused slight sodium and urine retention in acute experiments on hydrated rats.



In acute work on more than 100 anæsthetized, normotensive cats and dogs, intravenous injections of *SRG* 95213 (2·0–10·0 mgm./kgm.) caused an immediate and prolonged fall in arterial blood pressure. In contrast, benzothiadiazine diuretics, in doses as high as 40·0 mgm./kgm., produced little or no change in blood pressure under the same experimental conditions. In chronic work on hypertensive dogs (Goldblatt renal preparations) and rats (acti[illegible]deoxycorticosterone acetate and metacorticoid hypertension), daily oral administration of 5·0–20·0 mgm./kgm. of *SRG* 95213 resulted in a gradual and sustained reduction in blood pressure followed by a gradual recovery to pre-treatment-levels after the drug was discontinued (Figs. 1 and 2).

In other experiments designed to elucidate the mechanisms whereby this agent produces its characteristic effects, it was observed that *SRG* 95213-induced hypotension in anæsthetized cats was not blocked by previous administration of atropine, phentolamine, hexamethonium, chlorpheniramine, reserpine or by transection of the cervical spinal cord. In addition, direct injections of *SRG* 95213 into the femoral, renal and coronary arteries of anæsthetized dogs caused immediate and prolonged increases in the rate of flow of blood through each vascular bed. The results of this work suggest that *SRG* 95213 does not primarily influence nervous pathways or humoral factors in producing its hypotensive effect, but rather induces a direct peripheral vascular relaxation. *In vitro* experiments on rat and rabbit aortic strips tend

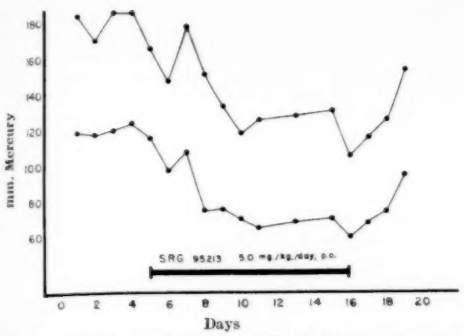


Fig. 1. Anti-hypertensive effect of *SRG* 95213 on the systolic and diastolic pressures of one renal hypertensive dog

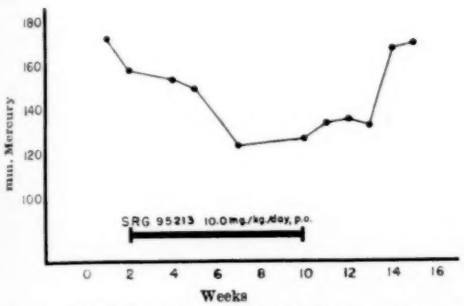


Fig. 2. Anti-hypertensive effect of *SRG* 95213 on the mean systolic blood pressure of 9 DOCA hypertensive rats

to support this thesis by demonstrating *SRG* 95213 antagonism to contractions induced by several different classes of vasoactive agents, for example, norepinephrine, angiotensin and serotonin.

In summary, it has been shown that *SRG* 95213, although structurally similar to the benzothiadiazine diuretics, is, in fact, non-diuretic but does reduce blood pressure in normotensive as well as hypertensive animals. Preliminary clinical observations in hypertensive individuals have, so far, confirmed the results obtained with animals. It is therefore not unreasonable to assume that *SRG* 95213 represents a true separation of the anti-hypertensive and diuretic properties associated with benzothiadiazine diuretics. The possibility does exist, however, that *SRG* 95213 possesses an anti-hypertensive action unlike that of the benzothiadiazine diuretics and, therefore, may be representative of a new class of anti-hypertensive agents.

Alan A. Rubin
Franklin E. Roth
Martin M. Winbury

Department of Pharmacology,
Biological Research Division,
Schering Corporation,
Bloomfield, New Jersey.

[1] Tapia, F. A., Dustan, H. P., Schneckloth, R. E., Corcoran, A. C., and Page, I. H., *Lancet*, ii, 831 (1957).
[2] Frein, E. D., Wanko, A., Wilson, I. M., and Parrish, A. E., *Ann. N.Y. Acad. Sci.*, **71**, 450 (1958).
[3] Hollander, W., Chobanian, A. V., and Wilkins, R. W., *Circ.*, **19**, 827 (1959).
[4] Preziosi, P., Bianchi, A., Loscalzo, B., and De Schaepdryver, A. F., *Arch. internat. pharmacodyn.*, **118**, 467 (1959).
[5] Rubin, A. A., Beauregard, S. C., and Freeman, B. G., *Fed. Proc.*, **19**, 101 (1960).

RADIOBIOLOGY

Effect of γ-Radiation on *Escherichia coli* grown in Deuterium Oxide Medium

It has been shown by several authors, for example by Alper and Gillies[1], that the nature of the growth medium has an effect on the radiation sensitivity of bacteria with regard to their ability to divide and form colonies. A simple, and yet considerable, change in the growth medium is the substitution of D_2O for H_2O. It was found that, on transferring cells from normal minimal medium (NH_4Cl 2 gm., Na_2HPO_4 6 gm., KH_2PO_4 3 gm., NaCl 3 gm., $MgCl_2$ 10 mgm., Na_2SO_4 26 mgm., glucose 5 gm. per litre) to the same made in 99·5 per cent D_2O, the cells grew, after a long lag period, with a generation time of 4 hr. at 37° C., which is about five times the normal. Similar results were found by Borek and Rittenberg[2]. Such cells, after growth, must possess very considerable substitution of deuterium for hydrogen, with consequent modification of hydrogen-bonded molecular structures as well as any changes due to the increased rotational moment of inertia of water. The greatly reduced rate of division could be due to slowed enzyme kinetics, to less efficient protein synthesis, or to some modification in the DNA, among other reasons. Since it is likely that radiation action, as regards cell division, is concerned with the DNA or its organization, it seemed of interest to see whether cells grown in D_2O showed any differences in radiation sensitivity.

The results of two separate experiments, in which cultures grown in H_2O and D_2O were simultaneously irradiated in a cobalt-60 source, are shown in Table 1.

Table 1

Dose (r.)	Survival (per cent) D_2O First experiment	Survival (per cent) H^2O	Survival (per cent) D_2O Second experiment	Survival (per cent) H_2O
5,000	15·0	8·0	28	23
10,000	4·8	2·1	5·1	3·9
15,000	1·5	$3·7 \times 10^{-1}$	0·7	1·1
20,000	$2·1 \times 10^{-1}$	$1·6 \times 10^{-1}$	$1·1 \times 10^{-1}$	0·26
25,000	$2·5 \times 10^{-2}$	$3·5 \times 10^{-2}$	$3·7 \times 10^{-2}$	$1·1 \times 10^{-1}$
30,000	$7·1 \times 10^{-3}$	$1·1 \times 10^{-2}$	$4·4 \times 10^{-2}$	$2·8 \times 10^{-3}$

Both types of bacteria are inactivated, to a first approximation, according to the relation $\ln(n/n_0) = -(\text{constant})\,(\text{dose})$, when n is the number of surviving colony-forming units for a given dose, and n_0 is the number present for no dose. The average 37 per cent doses, for which $\ln(n/n_0) = -1$, are 3,040 r. for H_2O bacteria and 3,230 r. for D_2O. The difference is not significant.

In these experiments the bacteria were diluted in H_2O medium and plated on H_2O agar. Since the return transition to H_2O might be thought to affect the colony count, the colony counts so found were checked against the turbidity of the culture, which is measured in D_2O. The plate counts matched the expected turbidity. Microscopic observations showed no visible alteration in the bacteria grown in D_2O. It is believed, therefore, that the colony counts made in this way are significantly related to survival. Whether cells plated on D_2O agar would show a difference cannot be said here.

In addition to these experiments the relative radiation sensitivity while the cells were adapting to D_2O was measured, and the results are shown in Table 2.

These results involve separate observations from the previous series. The variation in D_{37} is probably only

Table 2. Relative Radiation Sensitivity of Bacteria while adapting to D_2O

Time (hr.)	Control colony count	D_{37} (roentgens)
0	$1{\cdot}4 \times 10^8$	3,000
16	$1{\cdot}8 \times 10^8$	4,100
23	$1{\cdot}6 \times 10^8$	6,000
39	$9{\cdot}0 \times 10^8$	4,900
47	$4{\cdot}3 \times 10^7$	3,700

significant as regards the 23-hr. stage, just before the cells began to grow and divide in the new medium. This kind of behaviour has already been reported for normal cells by Stapleton[3] and cannot be ascribed to any special effect in D_2O.

These experiments suggest that the DNA in deuterium-grown bacteria is not markedly different, using radiation sensitivity as an indicator, from that in normally grown bacteria. The considerable modification in growth-rate, therefore, still offers a challenge in interpretation.

Acknowledgement is made of the assistance of Mrs. Anne Porter in the bacterial assays.

The work was aided by the U.S. Atomic Energy Commission.

Ernest Pollard

Department of Biophysics,
Yale University.

[1] Alper, T., and Gillies, N. E., *J. Gen. Microbiol.*, **18**, 461 (1958).
[2] Borek, E., and Rittenberg, D., *Proc. U.S. Nat. Acad. Sci.*, **46**, 777 (1960).
[3] Stapleton, G. E., Ph.D. thesis, Univ. of Tennessee (1952).

Specificity of Plant Feeding in Mosquitoes as determined by Radioactive Phosphorus

The development of resistance to insecticides in mosquito populations has made it increasingly apparent that alternative methods of control are required. In this connexion, the plant-feeding habits of mosquitoes might offer a possible approach to control by biological means. If specificity could be proved in the plant-feeding habits of any mosquito species, the extermination of such plants might lead to the control of that species.

It is generally assumed in entomological literature that mosquitoes, especially the males, feed on nectar from flowers. In work using phosphorus-32 on the feeding habits of arctic mosquitoes, West and Jenkins[1] claimed that, under laboratory conditions, both males and females of *Aedes communis* DeGeer ingested the juices and nectar from flowers of several species of arctic plants. The present communication gives results of preliminary experiments, done in the Biology Branch of Atomic Energy of Canada, Ltd., Chalk River, Ontario, to investigate the plant-feeding habits of *Aedes aegypti* L. reared in the laboratory and of native *Aedes* species from this area.

In these experiments, the flowers and leaves of 24 different species of native plants were labelled with phosphorus-32 solutions at a concentration of 20 μc./ml. Preliminary tests with cotton wicks, using different concentrations of phosphorus-32 (as $H_3{}^{32}PO_4$) in 5 per cent dextrose solution, had proved that this concentration gave easily detectable counts in both male and female mosquitoes, which fed on the dextrose solution.

So far as possible, plants found in bloom around the breeding places of mosquitoes were used for the experiments. These were brought into the laboratory and their stems were cut short under water. The flowers and/or leaves of these plants were placed in 2-ounce bottles with the ends of the stems, wrapped in cotton, immersed in 30 ml. of phosphorus-32 solution. The mouths of the bottles were covered with cheese cloth to prevent the mosquitoes from coming in direct contact with the radioactive solution. Without exception, the flowers and leaves of these plants took up appreciable amounts of radioactivity, reaching in some species about 25,000 count/min./cm.² of leaf. However, the presence of radioactivity in the nectar was not specifically determined.

Larvæ of native mosquitoes were collected from temporary pools and swamps and reared together to the adult stage in the laboratory. Three species of *Aedes* were identified in these collections. These were *Aedes implacabilis* (Walker), *Aedes dianteus* H.D. and K., and *Aedes punctor* (Kby.). The native species were tested as a mixed population and separate tests were carried out with pure cultures of *Aedes aegypti*.

For each feeding experiment 30 adults, including both males and females approximately one to two days old, were removed from the stock emergence cage to small laboratory cages (about 11 in. × 11 in. × 26 in.) having sliding plastic fronts. These mosquitoes, previously fed on 5 per cent dextrose solution, were left for 24 hr. without food before the start of any experiment. After a 24-hr. period of confinement with the plants, the mosquitoes were killed, washed in three changes of distilled water, dried between filter papers, and mounted under cellulose tape on counting planchets. This method of mounting was also used for the plants where, in most cases, the phosphorus-32 uptake of whole flowers and unit samples of leaves (1 cm.²) were measured.

Counts of radioactivity were made with a Geiger-Müller end-window tube (3·1 mgm./cm.²) and a Mareeni scaler. All counts were corrected for background and decay of phosphorus-32. The plants used are listed in Table 1.

Table 1

Acer rubrum L.	*Fragaria americana* (Porter) Britton
Amelanchier bartramiana (Tausch) Roemer	*Geranium bicknelii* Britton
Amelanchier canadensis (L.) Medic.	*Osmunda cinnamomea* L.
Amelanchier laevis Vieg.	*Prunus depressa* Pursh.
Clintonia borealis (Ait.) Raf.	*Salix humilis* Marsh
Comptonia peregrina (L.) Coulter	*Spiraea latifolia* (Ait.) Borkh.
Corydalis sempervirens (L.) Pers.	*Streptopus roseus* Michx.
Corylus sp.	*Taraxacum officinale* Weber
Cypripedium acaule Ait.	*Trillium grandiflorum* Michx.
Epigaea repens L.	*Vaccinium angustifolium* Ait.
Erythronium americanum Ker-Gawl.	*Viburnum alnifolium* Marsh
	Viola conspersa Reichereb, J. E. Smith.

Results of these laboratory tests showed that feeding had taken place on only three species of plants, namely, *Viola conspersa*, *Trillium grandiflorum*, and *Spiraea latifolia*. All mosquitoes confined with these three plant species became radioactive; the average counts of the mosquitoes are shown in Table 2.

Table 2

	Aedes aegypti	Native *Aedes* sp.
Viola conspersa (American dog violet)	517 counts/min.	114 counts/min.
Trillium grandiflorum (white trillium)	315 ,,	8 ,,
Spiraea latifolia (meadow-sweet)	54 ,,	32 ,,

An important finding in these laboratory experiments was that, in all cases, the mosquitoes confined with flowers alone did not receive any radioactivity; on the other hand, placing leaves with the respective flowers of these three species of plants or the use of leaves only gave positive results. This evidence indicates that, under the conditions of these experiments, mosquito feeding was confined to plant juices from the leaves.

These results differ from those of West and Jenkins on *Aedes communis*[1] and emphasize the need for further work on the plant-feeding habits of mosquitoes.

We are indebted to Dr. Marshall Laird, Institute of Parasitology, McGill University, for suggesting the problem, to Dr. A. S. West, Zoology Department, Queen's University, for help in planning the experiments and identifying the mosquitoes, and to Dr. A. M. Moore, Biology Branch, Atomic Energy of Canada, Ltd., for identification of plants.

A. A. Abdel-Malek *
W. F. Baldwin

Biology Branch,
Atomic Energy of Canada Limited,
Chalk River,
Ontario.

* McGill University Postdoctoral Fellow in Entomology. Permanent address: Department of Entomology, Faculty of Science, University of Cairo, Giza, Egypt.

[1] West, A. S., and Jenkins, D. W., *Mosquito News*, **11**, 217 (1951).

Effects of Irradiation on the Incorporation of Amino-Acid into Normal and Regenerating Rat Liver

The biosynthesis of proteins has been studied in normal rat liver[1,2] and in regenerating liver of partially hepatectomized rats[3]. Although the effect of ionizing radiations on the incorporation of amino-acids into proteins in normal rat liver has been investigated[4,5], to our knowledge it has not been investigated in regenerating rat liver. The present report presents a comparison of the effects of X-rays on the potential capabilities of livers of normal and partially hepatectomized rats to incorporate amino-acids into their liver proteins in relation to the dose and time of exposure to radiation after hepatectomy.

In the first of two series of experiments, 20 Sprague Dawley rats, each weighing 80–100 gm., were used. Three groups, each consisting of 5 rats, were exposed to 500, 1,000, or 2,000 r. of total-body X-irradiation, whereas the remaining five rats served as the non-irradiated control group. In the second of these series, 20 rats, each weighing 200–250 gm., were partially hepatectomized[6] under ether anæsthesia. Four groups, each consisting of four rats, were given total body exposures of 1,000 r. 2, 4, 6, and 18 hr. after hepatectomy, whereas the remaining hepatectomized animals served as the non-irradiated control group. In both series of experiments the radiation was delivered by an X-ray tube operated at 250 kV. and 15 mA. The radiation was filtered through 0·5 mm. of copper and the radiation field was rendered uniform by means of a parabolic mirror, measuring in depth 14·2 cm. at the centre. The dose-rate was 50 r./min. All rats were starved after X-irradiation, but had free access to water. In the second series, food was withdrawn after hepatectomy.

Each rat in the first series of experiments received an intraperitoneal injection of $3{\cdot}6 \times 10^6$ counts per min. (c.p.m.) (3·0 μmoles) of leucine-U-^{14}C 3 hr. after X-irradiation, whereas the rats in the second series received 6·0 μmoles of leucine-U-^{14}C 22 hr. after operation. All animals were killed 3 hr. after the injection. From each animal, 3 gm. of liver were removed and homogenized in a Dounce all-glass homogenizer at 0° C. for 3 min. in 15 ml. of a 0·35 *M* sucrose medium containing 0·025 *M* potassium chloride, 0·035 *M* potassium bicarbonate and 0·004 *M* magnesium chloride. Each homogenate was centrifuged at 105,000*g* at 0° C. for 2 hr. in a preparative 'Spinco' ultracentrifuge and the clear supernatant fraction was removed and filtered through 8 layers of fine cheese cloth. To 1·0 ml. of the supernatant fraction 5·0 ml. of 5 per cent trichloracetic acid (TCA) was added and the resulting precipitate sedimented by centrifugation was washed twice with 5 per cent TCA, alcohol and ether respectively, as described by Devi *et al.*[7]. In a few instances, the precipitate was heated at 90° C. for 15 min. in 5 per cent TCA, then washed and counted as before. The isotope concentration of the precipitate was not changed by this manipulation. Radioactivity was determined with a windowless gas-flow counter using aluminium planchets 12·5 mm. in diameter on to which an infinitely thin layer (less than 5 mgm.) of the aforementioned precipitate had been placed. The counting error did not exceed 3 per cent.

Table 1. Incorporation of Leucine-U-^{14}C into Liver Proteins of Normal and Partially Hepatectomized Irradiated Rats

Experimental series	Radiation dose (r.)	Time of X-irradiation after hepatectomy (hr.)	^{14}C activity (c.p.m./mgm. of protein)	
			Range of values	Mean of values ± *S.E.*
Non-hepatectomized rats (Series I)	0	—	400–600	510 ± 37
	500	—	460–650	570 ± 36
	1,000	—	500–700	606 ± 39
	2,000	—	550–800	680 ± 46
Hepatectomized rats (Series II)	1,000	2	600–780	702 ± 30
	1,000	4	640–760	708 ± 22
	1,000	6	650–710	686 ± 14
	1,000	18	790–1,050	938 ± 44
	0	—	860–1,120	1,018 ± 45

The results of both series of experiments are summarized in Table 1. Evidently the intact rat liver differs qualitatively from the hepatic tissue remaining after partial hepatectomy in so far as the effect of X-irradiation on the incorporation of leucine-U-^{14}C into liver proteins is concerned. Thus X-irradiation at 1,000 or 2,000 r. is followed by an increase in the isotope concentration in the proteins of the non-hepatectomized rat liver, whereas in the case of regenerating liver exposed to 1,000 r. a decrease in the isotope concentration in liver proteins is found if the animals are irradiated within 6 hr. after hepatectomy. After this time period, the exposure of the hepatectomized rats to X-rays is without effect on amino-acid incorporation. The time period after hepatectomy during which the partially hepatectomized liver exhibits evidence of radiosensitivity is probably not characterized by a change in cell number since it precedes that period during which the DNA concentration increases, a pre-requisite condition for an increased rate of mitosis[8]. It is conceivable that during the first 6 hr. after hepatectomy conditions exist which either limit the supply of amino-acid-activating enzymes or of enzymes required for transfer of the activated amino-acids to microsomal ribonucleic acid and protein. Either condition would reduce the rate of utilization of amino-acids for protein synthesis. In interpreting the significance of the observed changes in the hepatectomized, X-irradiated liver, it must be remembered that there are several uncontrolled variables in the experimental conditions prevailing here: for example, the variation in elapsed time between X-irradiation and the injection of ^{14}C-leucine, and the effect of starvation on the irradiated as compared with that on the non-irradiated rat livers. The contribution of these factors is probably small compared with that of the X-irradiation.

In the non-hepatectomized rat, radiation-induced changes are limited to the 1,000 r. and 2,000 r. dose-level; at the dose-level of 500 r. the increase is not statistically significant. Although the mechanism underlying the increased incorporation of carbon-14 into the proteins of normal livers of the irradiated animals remains unknown, two possible factors may be operating: (*a*), a contraction of the leucine pool may occur, thus reducing the isotope dilution of the injected labelled leucine, and/or (*b*), a translocation of ^{14}C-labelled protein from radiosensitive sites to the less sensitive hepatic tissue[9].

In conclusion it may be stated that the normal rat liver, with cells in the inter-mitotic phase, and regenerating liver, many cells of which are in the pre-mitotic phase, seem to respond differently to the action of ionizing radiation in so far as their ability to incorporate ^{14}C-labelled leucine into protein is concerned.

We thank Mr. K. I. Altman for his interest in this work and for his many helpful suggestions.

This work was supported, in part, by a grant from the United States Atomic Energy Commission.

Nirmal K. Sarkar*
Anima Devi*
Louis H. Hempelmann
University of Rochester School of Medicine, Rochester, New York.

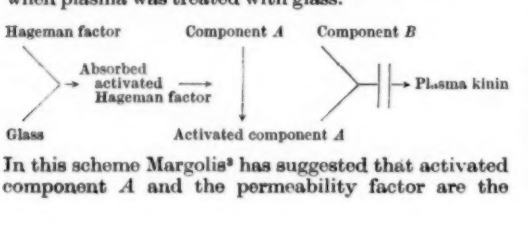
* Present address: Faculty of Medicine, Laval University, Quebec, Canada.

[1] Zamecnik, P. C., and Keller, E. B., *J. Biol. Chem.*, **209**, 337 (1954).
[2] Simkin, J. L., and Woru, T. S., *Biochem. J.*, **67**, 617 (1957).
[3] Von der Decken, Alexander, and Hultin, T., *Exp. Cell Res.*, **14**, 88 (1958).
[4] Holmes, B. E., and Mee, L. K., *Brit. J. Radiol.*, **25**, 273 (1952).
[5] Richmond, J. E., Ord, M. G., and Stocken, L. A., *Biochem. J.*, **66**, 123 (1957).
[6] Higgins, G. M., and Anderson, R. M., *Arch. Path.*, **12**, 186 (1931).
[7] Devi, Anima, Lerman, Sidney, and Sarkar, Nirmal K., *Nature*, **190**, 1193 (1961).
[8] Kelly, L. S., Hirsch, J. D., Beach, G., and Palmer, W., *Cancer Res.*, **17**, 117 (1957).
[9] Haberland, G. L., Schreier, K., Altman, K. I., and Hempelmann, L. H., *Biochim. Biophys. Acta*, **25**, 241 (1957).

HÆMATOLOGY

Role of Hageman Factor in the Activation of Vasodilator Activity in Human Plasma

Hageman factor is a clot-producing substance which is not found in the plasma of patients with Hageman trait[1]. The coagulation of blood obtained from patients with Hageman trait is grossly abnormal, although hæmostasis is usually unimpaired. The defect in blood in Hageman trait may be corrected *in vitro* by the addition of partially purified Hageman factor[1,2]. Margolis[3,4] has shown that Hageman factor is required for the appearance in glass-treated human plasma of a permeability factor and of a pain-producing and smooth muscle-contracting substance believed to be a plasma kinin. Three separate stages have been proposed for the release of plasma kinin when plasma was treated with glass.



In this scheme Margolis[3] has suggested that activated component *A* and the permeability factor are the same substance. Since the permeability factor and the glass-activated proteinase (activated component *A*) have not been distinguished clearly from plasma kallikrein[5], work was undertaken to determine whether Hageman factor might be related to the activation of plasma kallikrein. Conceivably, component *A* might be identified with kallikreinogen which on activation becomes plasma kallikrein. In turn, plasma kallikrein could act through component *B* to release kallidin from kallidinogen[6].

Citrated plasma from patients deficient in Hageman factor and plasma from normal individuals were prepared in silicone-coated tubes and kept frozen until used. 'Celite' activation was accomplished by the addition of 1–2 mgm. 'Celite Filter-cel' (Johns Manville)/ml. of plasma and gentle agitation for 10 min. at 25°; acetone activation was accomplished by the addition of 0·25 ml. of acetone/ml. of plasma and by standing at 25° for 4–6 hr.; and trypsin activation by the addition of 1 mgm. crystalline trypsin/ml. of plasma and by standing at 25° for 15–20 min.[7]. The vasodilator activity in the preparations, a measure of kallikrein activity, was determined by measuring the increase in blood-flow following their injection into dogs[5]. The results in Table 1, recorded in Frey units of human plasma kallikrein, are averages of two or more determinations. The vasodilator activity obtained by all methods of activation was probably mediated through a proteinase, since the addition of soybean trypsin inhibitor after activation prevented the increase in blood-flow.

'Celite' induces clot-promoting activity in plasma containing Hageman factor but lacks this effect in plasma deficient in this substance[8]. In these experiments, 'Celite' released small quantities of vasodilator activity from normal plasma. With plasma deficient in Hageman factor, no activation occurred. Similarly, vasodilator activity did not appear in Hageman factor-deficient plasma treated with acetone, a technique thought to destroy inhibitors and thus permit the activation of kallikreinogen by kallikreinogenase. Trypsin activation, on the other hand, is thought to attack the kallikreinogen molecule directly. This method liberated vasodilator activity both in normal and in Hageman factor-deficient plasma. The addition of partially purified Hageman factor[9] to the Hageman factor-deficient plasma gave it the ability to produce vasodilator activity upon treatment with 'Celite' or acetone. The preparation of Hageman factor contained approximately 1·5 times as much Hageman factor as that found in 1 ml. of plasma and was deficient in other recognized clotting factors and in plasminogen, from which Hageman factor is readily separable[1,10,11]. Higher concentrations of activated Hageman factor than those used in this work were capable of producing vasodilator activity in dogs presumably by activation of the dogs' plasma kallikrein. However, the results varied from one dog to another when compared with the response obtained with human plasma kallikrein and did not appear to give the same dose-response relationship.

Table 1. Effect of Hageman Factor on the Release of Vasodilator Activity in Human Plasma

Method of activation	Frey units of plasma kallikrein activated in: Normal plasma	Hageman plasma	Hageman plasma + Hageman factor*
None	< 0·03	< 0·03	< 0·03
'Celite'	0·13	< 0·03	0·5
Acetone	6·1	< 0·6	9·3
Trypsin	10·7	5·4	—

All results calculated per ml. of starting plasma.
* Partially purified Hageman factor gave no detectable vasodilator activity with acetone activation, and < 0·15 Frey unit of activity with 'Celite' activation.

These experiments are compatible with the hypothesis that the plasma of patients with Hageman trait is not deficient in kallikreinogen but lacks either kallikreinogenase itself or some prior enzymic step required for its activation by acetone or 'Celite'. Whether the vasodilator activity released from human plasma by the various methods of activation is due to the release of a single proteinase cannot as yet be unequivocally stated. However, plasmin, because of its relatively low potency in producing vasodilatation[5], cannot be one of the proteinases involved. Similarly, the question of the identity of plasma kallikrein with the permeability factor and the glass-activated proteinase in human plasma cannot as yet be resolved. The results provide evidence that a common mechanism is involved in their release, but this does not exclude the possibility that the enzymes themselves may be different. Certainly, however, the simplest hypothesis would be that they were the same substance. Keele[11] has reported that salivary and urinary kallikrein form kallidin in the absence of component *B* and in the presence of soybean trypsin inhibitor. It would be of interest to know whether plasma kallikrein, which is inhibited by soybean trypsin inhibitor, produces kallidin by a process dependent on component *B*.

This work was supported in part by grants from the National Heart Institute, National Institutes of Health, U.S. Public Health Service, and the American Heart Association.

M. E. Webster

National Heart Institute,
Bethesda, Maryland.

O. D. Ratnoff*

Western Reserve University School of Medicine
and University Hospitals of Cleveland,
Cleveland, Ohio.

*Career investigator, the American Heart Association.

[1] Ratnoff, O. D., and Colopy, J. E., *J. Clin. Invest.*, **34**, 602 (1955).
[2] Ratnoff, O. D., *Hageman Trait, Thrombosis et Diath. Hæmor.*, **4**, Supp. 116 (1960).
[3] Margolis, J., *Austral. J. Exp. Biol.*, **37**, 239 (1959).
[4] Margolis, J., *J. Physiol.*, **151**, 238 (1960).
[5] Webster, M. E., and Pierce, J. V., *J. Pharm. Exp. Therap.*, **130**, 484 (1960).
[6] Werle, E., *Polypeptides which Stimulate Plain Muscle*, edit. by Gaddum (E. and S. Livingstone, 1955).
[7] Werle, E., Forell, M. M., and Maier, L., *Arch. Exp. Path. Pharmakol.*, **225**, 369 (1955).
[8] Waaler, B. A., *Scand. J. Clin. Lab. Invest.*, **11**, Supp. 37 (1959).
[9] Ratnoff, O. D., Davie, E. W., and Mallett, D. L., *J. Clin. Invest.*, **40**, 803 (1961).
[10] Ratnoff, O. D., Hartmann, R. C., and Conley, C. L., *J. Exp. Med.*, **91**, 123 (1950).
[11] Keele, C. A., in *Polypeptides which affect Smooth Muscles and Blood Vessels*, edit. by Schachter (Pergamon Press, 1960).

Presence of Two Iron-Transport Proteins in Serum

The iron-binding protein transferrin was isolated in crystalline state as early as 1952, and it has been generally accepted that this was the only iron-containing protein in serum under normal conditions.

Using electrophoresis in starch supporting medium according to Kunkel and Slater[1] and iron-59 determinations after electro-plating, it is possible to detect minor variations in the electrophoretic pattern which are not in agreement with this point of view. The experiments were performed on human beings and the results are given in Table 1.

When serum and ^{59}Fe are incubated for 15 min. and investigated by electrophoresis, 11·7 per cent of the ^{59}Fe is found in front of the transferrin containing β-globulin. It must be noted that no activity is found in the γ-globulin.

Table 1. Percentage of Total ^{59}Fe Activity

	Sample	Albumin + α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin
	In vitro:				
1	serum + ^{59}Fe	6·0	5·7	88·2	0·1
	In vivo:				
2	Serum + ^{59}Fe injected	2·6	3·3	94·1	0·0
3*a*	Serum + ^{59}Fe injected 15 min. after injection	5·5	1·3	93·2	0·0
3*b*	25 min. after injection	3·0	1·8	95·2	0·0
4*a*	Transferrin + ^{59}Fe 15 min. after injection	10·0	7·	81·3	1·5
4*b*	25 min. after injection	9·4	6·4	82·0	2·2
5*a*	^{131}I-transferrin Rabbit	0·1	0·6	84·8	14·5
5*b*	Human	0·2	0·0	97·2	2·6

When serum from a patient is incubated with ^{59}Fe and re-injected, the serum from the patient shows the same type of pattern. It is of interest to note that two samples taken after an interval of 10 min. show the same relative distribution although the activity has decreased by 25 per cent from the first to the second sample.

If the ^{59}Fe is incubated with transferrin (Behringwerke) we find again the same pattern with a splitting of the ^{59}Fe into more than one protein fraction.

The electrophoretic findings are in many ways identical with the effect of a protein–protein interaction. This could, however, be shown not to be the case. If iodine-131-labelled transferrin is injected into a rabbit or in a man, ^{131}I is found only at the expected place for transferrin and not where the fast fraction of the ^{59}Fe was found.

The results can therefore only be taken to indicate that serum contains a protein which is not identical with transferrin and which has the ability to bind ^{59}Fe reversibly.

The constant relative size of the fast fraction in experiments 4*a* and *b*, where the iron was injected bound to transferrin, makes it probable that this smaller fraction may even represent an intermediary stage between transferrin and the erythroblasts.

The presence of such an intermediate in serum has been suggested by the work of Najean, Ardaillou and Bernard[2]. They showed that the rate of iron transfer from transferrin to reticulocytes could be augmented by adding serum to the system. The active component was non-dialysable and thermolabile.

In the ghosts of erythrocytes from animals injected with ^{59}Fe Falbe-Hansen and Lothe[3] found an iron-containing fraction which could not be identified with known iron proteins. This fraction appeared to be an intermediate stage in iron transport as it could only be found during the first hours after the ^{59}Fe injection. The possibility of identity between these two fractions is being investigated.

M. Faber
R. Jordal

Finsenlaboratory,
Finseninstitutet og Radiumstationen,
Copenhagen.

[1] Kunkel, H. G., and Slater, R. J., *Proc. Soc. Exp. Biol. Med.*, **80**, 42 (1952).
[2] Najean, Y., Ardaillou, R., and Bernard, J., *Rev. Franç. Etud. Clin. Biol.*, **5**, 783 (1960).
[3] Falbe-Hansen, I., and Lothe, K., *Acta Physiol. Scand.* (in the press).

Genetical Evidence for Synthesis of Transferrin in the Fœtus

Clear-cut evidence that the fœtus synthesizes its own plasma proteins is scant. It has been demonstrated by *in vitro* experiments that the liver of a fœtus of three months gestation can incorporate glycine labelled with carbon-14 into proteins resembling albumin, α_1-globulin, α_2-globulin, and β-globulin of human plasma[1]. These investigations also showed that metal-binding β-globulin (presumably transferrin) was capable of being synthesized by fœtal liver. Other workers have established that the fœtus synthesizes haptoglobin by finding some new-born children with types of haptoglobin that were absent in their mothers[2,3].

In the course of a survey of 212 cord blood sera by starch-gel electrophoresis, genetical evidence was obtained indicating that the fœtus produces its own transferrin.

The sera, each with sufficient hæmoglobin added to saturate the haptoglobin completely, were tested by Smithies's technique of starch-gel electrophoresis[4], using Poulik's discontinuous buffer system[5]. The resultant gels were sliced horizontally, one half stained with amido black 10*B* to identify protein bands, and the other half with a dilute benzidine stain to locate the haptoglobin–hæmoglobin complexes[6].

Three of the 212 cord sera contained two protein bands in the transferrin region. The faster migrating of these two bands corresponded to the usual transferrin designated as *C*. In each case, the mobility of the slower-migrating band was identical to that of the D_1 transferrin of a reference standard. These three new-born children were therefore of the heterozygous transferrin type C/D_1. The remaining 209 cord blood sera contained only one band in the transferrin region, which had the mobility of *C* transferrin. These newborns were considered to be homozygous (*Tr C/C*). Two of the *Tr* C/D_1 new-born children were negroes, while the third was of Caucasian ancestry. Other examples of slow-migrating transferrins (which as a group are designated '*D*' transferrins) have been found in negro populations and Australian aborigines[6]. The three mothers of the *Tr* C/D_1 new-born children had the usual, homozygous transferrin type (*Tr C/C*), while their husbands were all heterozygous (*Tr* C/D_1) (Fig. 1). In one of the negro families and in the Caucasian family, autoradiography with iron-59 performed[7] on fresh sera of the infants and their parents verified that the presumptive transferrin bands were truly iron-binding. Since each of the three mothers lacked the D_1 transferrin, it is evident that transplacental passage from mother to fœtus could not account for the D_1 transferrin in the cord blood, and consequently these new-born children must have synthesized the D_1 transferrin themselves.

We thank Dr. Eloise Giblett, of Seattle, Washington, for supplying us with *Tr* C/D_1 and *Tr* C/D_1 reference standard sera. This work was supported by grants from the John A. Hartford Foundation, Inc., and the National Heart Institute (*H*–4706).

A. R. Rausen
P. S. Gerald
L. K. Diamond

Department of Pediatrics,
Harvard Medical School, and
Department of Medicine,
Children's Hospital Medical Center,
Boston.

[1] Dancis, J., Braverman, N., and Lind, J., *J. Clin. Invest.*, **36**, 398 (1957).
[2] Galatius-Jensen, F., *Proc. Sixth Europ. Cong. Hæmat.*, 269 (1957).
[3] Rausen, A. R., Gerald, P. S., and Diamond, L. K., *Nature*, **191**, 717 (1961).
[4] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[5] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[6] Smithies, O., *Adv. Protein Chem.*, **14**, 65 (1959).
[7] Giblett, E. R., Hickman, C. G., and Smithies, O., *Nature*, **183**, 1589 (1959).

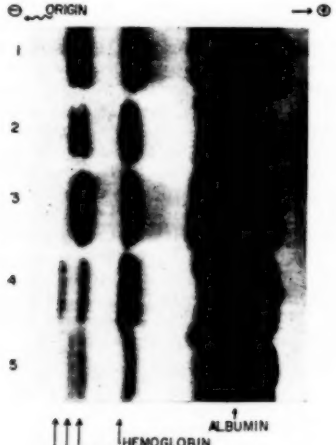


Fig. 1. Portion of starch-gel electrophoretic patterns of family with new-born of *Tr* C/D_1 pattern, run in parallel with reference standards. 1, New-born, *Tr* C/D_1; 2, reference standard, *Tr* C/D_1; 3, mother, *Tr C/C*; 4, reference standard, *Tr* C/D_2; 5, father, *Tr* C/D_1

IMMUNOLOGY

Rapid Preparation of Fluorescent Antibodies using Gel-Filtration

In the preparation of fluorescent antibodies the excess dye is generally removed by dialysis for 5–6 days (ref. 1). In order to avoid a time-consuming dialysis and to decrease the risk of destroying antibody activity we have used gel-filtration[2] in the preparation of a number of fluorescent antibodies, the titres of which were afterwards compared with those of dialysed samples. A preliminary report of the method has already been published[3].

After the conjugation of serum or a serum fraction with fluorescein isothiocyanate (Sylvana Chemical Co., Orange, New Jersey) as described by Riggs *et al.*[4], one portion was dialysed for six days at 2° C. against a saline-phosphate buffer (0·15 *M* sodium chloride in 0·01 *M* phosphate, *p*H 7·0) with two daily changes, and another portion was subjected to gel-filtration. One ml. of fluorescein-treated material was put on a 2 × 5 cm. column of gel-filter substance 'Sephadex *G*–25' (AB Pharmacia, Uppsala, Sweden), previously washed with the saline-phosphate buffer. During elution, which was performed with the same buffer, two yellow zones appeared. The fast-moving one contained the conjugated proteins, while the unbound dye was retarded partly by the gel-filtering effect and partly by adsorption to the 'Sephadex'. The strongly coloured protein fraction was collected and used for the subsequent comparison with the material prepared by dialysis. Generally, a two-fold dilution

Table 1. Effect on Antibody Activity of Dialysis and Gel-Filtration

Experiment No.		Antibody titres (dilution × observed titre)			
		Original serum or serum fraction	Fluorescein-labelled material		
			Without further treatment	Gel-filtered fraction	Dialysed fraction
1	Anti-*A* in pooled group *O* serum I	1 : 48	1 : 48	1 : 48	1 : 12
	II	1 : 16	1 : 12	1 : 12	
2	Separated 19 *S* anti-*A* I	1 : 128	1 : 80	1 : 72	1 : 80
	II	1 : 128	1 : 80	1 : 72	
3*a*	7 *S* anti-*D* I	1 : 128	1 : 160	1 : 160	1 : 20
	II	1 : 256	1 : 80	1 : 80	
3*b*	7 *S* anti-*D* III	1 : 128	1 : 20	1 : 36	No detectable activity
4	Rabbit anti-human γ-globulin I	1 : 3200	1 : 2000	1 : 2000	1 : 4000
	II	1 : 6400	1 : 8000	1 : 8000	

I, Titrated immediately after gel filtration; II, titrated after preparation of the dialysed fraction; III, titrated two days after completion of the dialysis.

Anti-*A* activity was determined using a 2 per cent suspension of type A_1 red cells in saline. In experiment 1 cells from different donors were used in I and II, which precludes direct comparison between the two series. In the other experiments I and II were titrated under similar conditions using fresh cells from the same donors.

Anti-*D* activity was titrated using a 3 per cent suspension of papainized group *O D*-positive red cells in saline.

Antiglobulin activity was determined on slides using a 5 per cent suspension in saline of four times washed group *O D*-positive red cells previously incubated with the anti-*D* serum used in experiment 3.

The dilution compared with the original serum or serum fraction was 1 : 2·5 for the fluorescein-labelled material without further treatment and for the dialysed fraction, and 1 : 4·5–1 : 6·25 for the gel-filtered fraction.

occurred during the passage through the column and, as expected, the *p*H of the alkaline protein solution changed from 9·0 to 7·0. The elution was usually completed in 10–15 min. Antibody titres were determined immediately after gel-filtration as well as after storage for 6 days at 2° C.–4° C., when the dialysed samples were also titrated. For comparison, on both occasions the original serum or serum fraction was titrated as well as the fluorescein-labelled material without removal of excess dye.

The following antibodies were investigated:

(1), Anti-*A* in pooled serum from three group *O* donors. (2), Anti-*A* separated from pooled human sera by starch-block electrophoresis[5]. The anti-*A* active fraction behaved as a 19 *S* γ-globulin in density gradient ultracentrifugation[6] and on diethylaminoethyl-cellulose (DEAE)[7]. (3*a*) and (3*b*), Anti-*D* of the incomplete type in the serum from an Rh-immunized woman. The antibody behaved as a 7 *S* γ-globulin in ultracentrifugation and on DEAE-cellulose[7]. (4), Rabbit anti-human γ-globulin No. 72 from Behringwerke A.G., Marburg, Germany. (5), Neutralizing antibody against chicken erythroleukæmia virus (CEV). Convalescent serum was obtained from a cock which had survived infection with CEV and had then been further inoculated subcutaneously four times with CEV at intervals of about one month. The serum was collected 48 hr. after the last CEV dose had been given. The globulin fraction used was precipitated by adding ammonium sulphate to half saturation.

The results of the titrations are presented in Table 1, which shows that no significant loss of activity was caused by the coupling with fluorescein isothiocyanate. Nor did any significant changes in titre occur during the later removal of excess dye by either gel-filtration or dialysis in the experiments with anti-*A* (1 and 2) or anti-human γ-globulin (4). Anti-*D* (3 *a* and *b*), however, was less stable, and some loss of activity occurred during storage of the fluorescein-treated material. Compared with the fluorescein-treated starting material the gel-filtered preparation showed no significant decrease in titre, whereas the dialysed fraction had a lower activity.

In the experiments with CEV antibody, blood smears from a chicken with erythroleukæmia were fixed with methanol for 10 min., stained for 30 min. with the fluorescent antibody solutions to be compared, and washed with saline-phosphate buffer. With this procedure the stem-cells (erythroblasts) showed a specific fluorescence in the cytoplasm, whereas other cells belonging to the red cell series were not stained. No qualitative difference was observed between the dialysed and the gel-filtered preparations. The staining properties of various other gel-filtered conjugates containing other antibodies, as well as other fluorescent dyes, have been qualitatively equal to those prepared by dialysis.

In a separate semi-quantitative experiment, fixed blood smears were stained with serial two-fold dilutions of CEV antibody, half of each slide being stained with a gel-filtered and the other half with a dialysed antibody solution of equal titre. The highest dilution at which fluorescence could still be seen was 1 : 64 for both preparations.

Thus it can be concluded that gel-filtration is superior to dialysis in the preparation of fluorescent antibodies mainly because of its extreme rapidity and also, to some extent, because it involves less risk of damage to labile antibodies.

This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

Johan Killander

Department of Clinical Chemistry,

Jan Pontén

Institute of Pathology I,

Lennart Rodén

Institute of Medical Chemistry,

University of Uppsala.

[1] Mellors, R. C., *Analytical Cytology*, second ed., 1 (McGraw-Hill Book Co., Inc., New York, 1959).

[2] Porath, J., and Flodin, P., *Nature*, **183**, 1657 (1959).

[3] Pontén, J., Reports from the Second Conference on Cell Research, Lund, 1960. *Kungl. Fysiografiska Sällskapets handlingar*, N.F., **71**, 51 (1960).

[4] Riggs, J. L., Seiwald, R. J., Bruckhalter, J. H., Downs, C. M., and Metcalf, T. G., *Amer. J. Pathol.*, **34**, 1081 (1958).

[5] Kunkel, H. G., and Trautman, R., *Electrophoresis*, edit. by Bier, M., 225 (Academic Press Inc., New York, 1959).

[6] Kunkel, H. G., *The Plasma Proteins*, edit. by Putnam, F. W., 1, 294 (Academic Press, Inc., New York, 1960).

[7] Peterson, E. A., and Sober, H. A., *The Plasma Proteins*, edit. by Putnam, F. W., 1, 105 (Academic Press, Inc., New York, 1960).

BIOLOGY

Fluctuations in Hormone Activity in Brachyura Larvæ

Although similarities between the control of some chromatophores in larval and adult Crustacea have been noted[1], relatively little is known of the presence of chromatophorotropic substances or their development in larval Brachyura. The three zoeal stages and one megalops stage of *Sesarma reticulatum*, reared from hatching to the first crab and beyond, in controlled laboratory conditions, provide an excellent source of material to test for the presence of a *Uca*

black-dispersing substance in all stages of larval development and to determine if the activity of this substance during larval development is associated with the larval moult and inter-moult periods.

Eye-stalks were dissected from ten zoeæ at the following intervals: first zoeæ: immediately after hatching, and thereafter at 12-hr. intervals up to 72 hr.; second zoeæ: 6 hr. after moulting, 18 hr., 30 hr., 42 hr., 54 hr. and 65 hr.; third zoeæ: 2 hr. after moulting, 13 hr., 25 hr., 37 hr., 44 hr. and 55 hr.; megalops: 12 hr. after moulting, 24 hr. and 48 hr. The eye-stalks were placed in a glass dish, the excess water removed with filter paper, and the tissues homogenized with a ground glass rod. The homogenate was then diluted in 0·5 ml. of filtered sea water which was boiled and centrifuged. The supernatant fluid was injected into 10 adult *Uca* from which both eye-stalks had been removed 12–14 hr. previously. Each adult *Uca* received 0·05 ml. of the extract. Using the chromatophore index in which the number 5 indicates complete dispersal of the *Uca* black chromatophores and 1 signifies complete contraction[2] the chromatophore stage of the adult crabs was determined at intervals of 15, 30, 45, 60, 90, 120 and 150 min. after injection. The sums of the activities during the total period of dispersion[3] for the intervals noted previously were then compared to determine if fluctuations in hormone activity of the tracts were associated with a physiological state within the moulting cycle of the larvæ.

To compare possible differences in technique between successive experiments, eye-stalk extract from adult *Uca* (10 pairs/2·25 ml.) was injected into a separate series of 10 de-stalked *Uca* at the same time as the larval eye-stalks were assayed. Sea water controls, 0·05 ml. per adult *Uca*, were used with each experiment. Initially, muscle from the abdominal segments of the larvæ was injected into a third series of test *Uca*. Larvæ in each experiment were taken from one egg-mass to avoid possible genetic differences and eliminate variations in size of the larvæ. Where possible, injections were made during the same period of the day in order to avoid diurnal differences in the sensitivity of the black chromatophore response of the recipient *Uca*.

The *Uca* black-dispersing substance was found in the unstalked eyes of the first zoeal stage and in the eyestalks of all remaining larval stages of *S. reticulatum*. Dispersal of the black chromatophores of test *Uca* was not observed following injection of abdominal muscle from the larvæ.

At the time of hatching the total activity of the hormone was lowest. During the next 36 hr. the activity increased, but was again reduced at the interval preceding the first moult. The activity increased during the inter-moult period, was reduced prior to, and following, the second moult, and increased again during the intermoult period of the third zoeal stage. This same pattern, lower activity before and after moulting followed by an increase in total activity, was observed for extracts of eye-stalks of the megalops. Zoeæ which were delayed in moulting normally showed a decrease in total activity at the time when moulting would be expected but, if ecdysis did not follow, the total activity of the hormone increased again.

The observed fluctuations in total activity of the hormone cannot be attributed solely to size of the moulting zoeæ since the main reduction in activity occurred prior to moulting, in other words, without any change in size. The differences do not show any association with changes in light or phases of the tide. It is conceivable, therefore, that the fluctuations in activity are associated with moulting or the physiological processes which precede and follow moulting. Although the eye-stalk of the adult Brachyura is known to contain the major site of production of the moult-inhibiting hormone it has not been established when these organs develop functionally or if they control moulting in the larval stages. Even if different hormones are responsible for the control of colour changes and the control of moulting, several hypotheses are possible on the basis of these results: (1), the *Uca* black-dispersing substance located within the eye-stalks of the larvæ fluctuates because of changes in titre associated with moulting but is not directly responsible for, nor involved in, the actual moulting process, or (2), if the endocrine organs within the eye-stalks of the larval stages are responsible for production or storage of several hormones, including the moult-inhibiting hormone, the reduction of the titre of the *Uca* black-dispersing substance may reflect the reduction in titre of the moult-inhibiting hormone also.

The reduced titre is followed by ecdysis, a subsequent increase in concentration of the moult-inhibiting hormone, and another larval intermoult period. This pattern of decreasing activity or titre prior to moulting and increasing activity after moulting would be repeated through all larval moults and carried over into the crab stages.

The assistance of Miss Joanne O'Connell is gratefully acknowledged. The work was supported by the Office of Naval Research, *NR* 104–194.

John D. Costlow, jun.

Duke University Marine Laboratory,
Beaufort, North Carolina.

[1] Broch, E. S., *Biol. Bull.*, **119**, 305 (1960).
[2] Hogben, L. T., and Slome, D., *Proc. Roy. Soc.*, B, **108**, 10 (1931).
[3] Sandeen, M. I., *Physiol. Zool.*, **23**, 337 (1950).

Axenic Cultivation of an Enchytraeid Annelid

The monoxenic cultivation of *Enchytraeus fragmentosus* Bell, 1959[1] (that is, the rearing, through successive generations in serial subculture, of this micro-annelid in the company of one[2,3] other species—in this case, *Escherichia coli*), was reported by us here[4] some months ago. At that time our next goal was its axenic cultivation (that is, growth and reproduction, generation after generation, in complete isolation from living organisms—or parts thereof—belonging to other species[2,3]). We can now report success over a period exceeding 2 months at this date, with indications that such cultivation can be sustained indefinitely.

After our earlier report[4], *E. fragmentosus* has continued to grow monoxenically with little difficulty and is now in its sixth set of serial cultures. For our first axenic cultures we used a few of the numerous worms in an old (231-day) monoxenic culture originating with one of the 3 fragments into which the initially monoxenized organism had divided[4].

Axenization was accomplished by rinsing the worms 3 times in sterile distilled water, followed by their transfer to 10 ml. antibiotic solution (1,000 units potassium penicillin *G* and 100 γ streptomycin sulphate per ml.); after 1·5 hr. the worms were moved to a second antibiotic rinse, and, 50 min. later, 7 were inoculated on to 1·5 per cent nutrient agar slants in 16-mm. test-tubes, one worm per tube; the following day, 20 hr. later, 5 active worms (2 had died) were transferred to individual test-tubes con-

taining the experimental medium. None of the 7 transiently occupied nutrient-agar slants developed bacterial colonies.

The experimental medium was initially biphasic: (1), a semi-solid base of 1·5 per cent nutrient agar, autoclaved in test-tubes 16 mm. wide, allowed to cool to 48° C., and then, while still liquid, supplemented with a sterile heated (lamb) liver extract (HLE), prepared, according to a strict recipe[5] of relatively mild heating (53° C. for 6 min.), from liver homogenized with equal parts (w/v) distilled water. The HLE was incorporated to give a 16× dilution, uniform dispersal being achieved by gentle shaking of each tube before the agar hardened as a slant. (2), A liquid phase of much smaller volume—HLE (diluted × 16), added later at 0·1 ml./tube. After inoculation with antibiotic-treated worms, the experimental tubes were kept almost horizontal until evaporation left negligible free liquid—then, on the 32nd day, they were placed upright.

Of the 5 tubes one showed contamination with *E. coli* on the third day; one gave no visible evidence of contamination until the 32nd day (with an unidentified organism—not *E. coli*—of undetermined origin); and the remaining 3, by all evidence to date, received, and continue to harbour, axenized worms. In 2 of these 3 tubes the isolated worms grew to large size and began to fragment on the 36th day, yielding, within 1–2 days, 8 and 9 pieces, respectively. Sub-cultures into the same medium were made on the 39th day with 5 of the offspring from one tube, one piece of worm/new tube. Other sub-cultures have been initiated since. Most F_1 pieces, whether left in the original tubes or sub-cultured, have recently reached adult size; and, so far, 3 worms of the former group and 1 of the latter have fragmented, the earliest, in an original tube, starting during the 29th day following the onset of first generation reproduction. F_2 worms are growing, and further F_1 fragmentation appears imminent.

To our knowledge, *Enchytraeus fragmentosus* is the first axenically grown annelid, being of further special interest as the only metazoon of extensive regenerative powers to be so maintained through successive generations. Methods for such axenic culture of a few arthropods (insects, with the exception of one microcrustacean) and nematodes have been known for from several to many years[3]; and of a rotifer for a few months[5]. HLE has now proved useful in work with members of three major invertebrate taxa: *Caenorhabditis briggsae*[3] (class Nematoda, phylum Aschelminthes); *Lecane inermis*[5] (class Rotifera, phylum Aschelminthes); and *E. fragmentosus* (class Oligochaeta, phylum Nematoda). For the axenic maintenance of the second and third of these, HLE has so far been a crucial ingredient in all media. The preparation used for *E. fragmentosus* was sterilized by Seitz-filtration, which, curiously enough, has consistently yielded HLE's inadequate for the rotifer, *L. inermis* (the latter growing only with HLE filtered through 'Millipore' membranes).

With *E. fragmentosus* under axenic cultivation on a complex organic (oligidic[2,3]) medium, one may now reasonably set out to determine this annelid's absolute nutritional requirements.

We thank the National Science Foundation for partial support, through grants *G*–6018, *G*–13138 and *G*–15034.

The technical assistance of D. JoAnne Ferral, Larry G. Harris and Douglas L. Getchell is gratefully acknowledged.

Note added in proof. *E. fragmentosus* is now in its tenth set of serial cultures. In certain tubes some or all worms show tumorous growth. So far this phenomenon has been observed only when enchytraeids have been trapped in liquid—as when an excess of moisture collects, or is introduced[6], as a meniscus at the base of the slant, or when a worm is transferred into water or HLE (3 days is the shortest time so far observed with slants; 2 days with water).

ELLSWORTH C. DOUGHERTY*
BJØRN SOLBERG†

Laboratory for Gnotobiotic Studies,
2428 Bancroft Way,
Berkeley 4,
California.

* Present address: Department of Nutrition, College of Agriculture, University of California, Berkeley 4, California.
† Present address: 5920 College Avenue, Oakland 18, California.

[1] Bell, A. W., *Science*, **129**, 1278 (1959).
[2] Dougherty, E. C., *Ann. N.Y. Acad. Sci.*, **77**, 27 (1959).
[3] Dougherty, E. C., in *Nematology, Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms*, edit. by Sasser, J. N., and Jenkins, W. R., 297 (Univ. North Carolina Press, Chapel Hill, N.C., 1960).
[4] Dougherty, E. C., and Solberg, B., *Nature*, **186**, 1067 (1960).
[5] Dougherty, E. C., Solberg, B., and Ferral, D. J., *Anat. Rec.*, **138**, 344 (1960); *Experientia*, **17**, 131 (1961).
[6] Ferral, D. J., Dougherty, E. C., and Holinka, C. F., *J. Parasitol.*, **47**, suppl., 33.

Life-cycle of an Acanthocephalan Parasite of the Eider Duck

ABOUT 190 species of Acanthocephala occur in birds, but the life-histories are known only in five species. In all known cases the life-cycle is indirect.

Thom and Garden[1] reported an outbreak of disease among eider ducks caused by acanthocephalan parasites, and Harrison[2] also found them in eider ducks in Orkney. Rayski[3] analysed the systematic position of these Acanthocephala and suggested that they are *Profilicollis botulus* (syn. *Polymorphus botulus*). In the Ythan estuary, these parasites are known to be responsible for a seasonal epidemic with high mortality every year; this is also known to occur in the Dutch island of Vlieland (Van den Broek, personal communication).

In 1960, 98 dead eiders (*Somateria mollissima* (L.)) were collected in the Ythan estuary, 48 of which were unfit for examination. Of the 50 dissected, all except two were found to be infected with 100–750 specimens of *P. botulus*. This suggests that the intermediate host must be common in the locality of the habitat of the duck and must constitute a large proportion of the diet of the eider.

Samples of a wide range of the food of the eider taken from the Ythan estuary (which is brackish) were examined but with no positive result. The material examined was as follows : *Hydrobia ulvae*, *Littorina littorea*, *Retusa alba*, *Mytilus edulis*, *Corophium volutator*, *Crangon vulgaris*, *Gammarus zaddachi-salinus*, *G. zaddachi-oceanicus*, *Marinogammarus marinus* and *Cancer pagurus*.

Petrochenko[4], in his monograph on Acanthocephala, quotes a paper by Belopolskaya in which she states that the intermediate host of *P. botulus* is *Gammarus locusta*. We therefore concentrated on searching for these larvæ in a large number of different species of *Gammarus* from the habitat frequented by eiders, but with no success. For several months attempts were made to infect gammarids obtained from the south coast of the Firth of Forth near Edinburgh. Collecting areas were chosen which were not known to be fre-

quented by eiders. Laboratory-bred gammarids were also used in these experiments. Gammarids greedily devoured both eggs and worms containing eggs. They did not become infested, though in some cases eggs were seen in the alimentary canal several days later; hatching had not taken place.

Eventually, a single specimen of the common shore crab *Carcinus moenas* was collected and fed with a worm containing a large number of eggs of *P. botulus*. Two days later the crab was dissected and about a dozen acanthors were seen in process of hatching in the alimentary canal. Eventually 10 crabs collected from the same area were fed with eggs and worms containing eggs. Five crabs died in the space of 3–5 weeks after infection and were found to contain large numbers of developing acanthellæ. One crab died 50 days after infection, showing a large number of acanthella stages. Four crabs were killed and examined 62 days after infection and two of these contained fully developed cysts containing acanthellæ (cystacanths) and two others had a large number of acanthellæ.

From these experiments it was clear that the crabs had been hyper-infected, and at the same time it was obvious that full development of *P. botulus* takes place in *C. moenas*.

Since then, 68 shore crabs from the Ythan estuary have been examined, and 29 of them were found to be infested with cysts containing acanthellæ of *P. botulus*, the numbers varying from 1 to 9 in each crab. When these were examined microscopically and the proboscis of the larvæ was compared with the proboscis of adults, it was found that number, arrangement and shape of hooks were identical. The cystocanths were situated in the region immediately under the heart, and either above the hind gut or on both sides of it. The very high rate of infestation in the shore crab in the Ythan estuary explains the common occurrence of this acanthocephalan parasite in eider duck.

This is the first record of the crab *Carcinus moenas* acting as intermediate host in the life-history of acanthocephalan parasites.

Unfortunately, Belopolskaya's paper is not available to us, and we have been unable to find out on what grounds she concluded that the larvæ found in *G. locusta* were really the early stages of *P. botulus*.

C. Rayski
E. A. Garden

Department of Zoology,
University of Edinburgh,
West Mains Road,
Edinburgh, 9.

[1] Thom, V. M., and Garden, E. A., *Fair Isle Bird Observ. Bull.*, **2** (7), 325 (1955).
[2] Harrison, J. N., *Bull. Brit. Ornith. Club*, **75** (9), 121 (1955).
[3] Rayski, C., *Proc. Fifteenth Int. Cong. Zool.*, 676 (1959).
[4] Petrochenko, V. I., *Acanthocephala of Domestic and Wild Animals*, **1** (Moscow, 1956); **2** (Moscow, 1958).

ENTOMOLOGY

Intracellular *Rickettsia*-like Micro-organisms in Certain Ticks

Little attention has been paid to the intracellular *Rickettsia*-like micro-organisms in acarines. Perhaps the first approach to a comprehensive survey on these organisms in ticks was that of Cowdry[1], although a few earlier observations were made[2–4]. Cowdry reported the occurrence of intracellular micro-organisms, of rickettsial nature, in the malpighian tubules and in eggs of several species of ixodid and argasid ticks. He pointed out that, being present in eggs and unfed larvæ, the organisms were transmitted by inheritance, and postulated a symbiotic function. Further work on the morphological appearances of such micro-organisms and their distribution in the malpighian tubules and oocytes of some of these tick species were developed by Buchner[5], Mudrow[6] and Jaschke[7].

Of the several species of *Argas*[8], however, it appears that only *A. persicus* has been shown to possess intracellular *Rickettsia*-like micro-organisms. Recent work[9] describes in detail for this tick the nature and distribution of the organisms in the malpighian tubules and in its oocytes at different stages of development; their recognition and description being based on histochemical methods and electron microscopy.

Suitor and Weiss[10] also recently reported mainly serological and cultural aspects of this organism, isolated from the malpighian tubules, but not, it appeared, from the oocytes, and proposed from their description the name *Wolbachia persica* S. and W.

The present communication reports on intracellular micro-organisms of similar rickettsial appearance in the ticks *Argas vespertilionis*, *A. transgariepinus*, and *A. boueti*, collected from bat-infested places in the Abu-Rawash area near Cairo, Egypt. In these ticks, as in *A. persicus*, the organisms are found in almost all cells of the malpighian tubules and in oocytes. In the malpighian tubule cells, the organisms are mostly present in groups of variable size, though single coccoid and short rod forms have been seen.

In the oocyte cytoplasm, the organisms appeared in smaller groups, and were only demonstrable after combined ribonuclease enzyme treatment and Giemsa staining[9] or after Feulgen–Schiff's technique.

Further work on the nature of the organisms in these species of *Argas*, including examination by electron microscopy is in progress; the work includes a survey also of representatives of other tick genera.

I wish to thank Prof. D. S. Bertram, director of this Department, for his valuable suggestions and helpful guidance in the work.

M. A. Roshdy

Department of Entomology,
London School of Hygiene and
Tropical Medicine,
Keppel Street, W.C.1.

[1] Cowdry, E. V., *J. Exp. Med.*, **37**, 431 (1923); **41**, 817 (1925).
[2] Stuhlmann, F., *Arb. Kaiserl. Gesundh.*, **26**, 301 (1907).
[3] Godoy, A., and Pinto, C., *Brasil Medico*, **2**, 335 (1922).
[4] Buchner, P., *Naturwiss.*, **33**, 1 (1922).
[5] Buchner, P., *Z. Morphol. Oekol. Tiere*, **6**, 625 (1926); *Gier und Planze in Symbiose*, **5**, 138 (Gebrüder Borntraeger, 1930).
[6] Mudrow, E., *Z. Parasitenk.*, **5**, 138 (1932).
[7] Jaschke, W., *Z. Parasitenk.*, **5**, 515 (1933).
[8] Hoogstraal, H., and Kaiser, M. N., *J. Egypt. Publ. Hlth. Assoc.*, **33**, (3), 51 (1958).
[9] Roshdy, M. A., *J. Insect. Path.*, **3**, (2), 148 (1961).
[10] Suitor E. C., and Weiss, E., *J. Infect. Dis.*, **108**, 95 (1961).

Biological Species confused under the Name *Culicoides austeni* (Carter, Ingram and McFie)

The age-grading techniques at present widely used in the study of biting Diptera having been found applicable to *Culicoides* in Gambia, they were used systematically with material referred to *C. austeni* (Carter, Ingram and McFie). As a result it

became apparent that material referable on morphological grounds to this species showed pronounced biological differences correlated with differences in its larval habitat. Hopkins[2] and Nicholas[3] reared this species from rotting banana litter and Gambian material from this habitat agreed with theirs in requiring blood-meals throughout life for maturation. It never mated under laboratory conditions and field observations showed that mating normally occurred in crepuscular swarms. Far more abundant in Gambia, however, was a form of this species which, while morphologically identical, differed in having its larval habitat in the salt-water mangrove swamps of the river estuary. The salt-water form proved to be autogenous in the first ovarian cycle, maturing its first batch of eggs at the expense of the larval fat body without taking a blood-meal. Swarming was never observed in this species and was evidently not obligatory since it mated freely without it under laboratory conditions, even as a single pair in a glass vial.

Since *C. austeni* s. str. was described from a coastal area (Accra, Ghana) the possibility of its being the salt-water form is quite strong. The soil-breeding form, presumably that studied by the Nigerian workers and implicated in transmission of filariasis, was synonymized with *C. milnei* Austen by Nicholas *et al.*[4]. *C. milnei*, however, also occurs in Gambia and is clearly distinct from both forms of *C. austeni* lat. in having fully separated eyes as stated by Carter *et al.*[1] in their original description of *C. austeni*.

Salt-water *C. austeni* exhibits a pronounced 14-day periodicity due to synchronization of emergence with spring tides. As a result of this, the age composition of the population varies cyclically, primiparæ biting for the first time, predominating at times of peak density and higher age groups at times of low density. Correlated with this rhythm, a marked decrease in survival-rate after the third oviposition was apparent when the dissection results were substituted into the total numbers. The immature stages have not been reared through from egg to adult, but enough is known to make it clear that the life-cycle cannot be completed between one spring tide and the next. With a hatching time of two days, a fourth instar larval life of 4–5 days and a pupal life of 3 days the probability is that it can be completed in two full tidal cycles. Taken together, these two factors imply the existence of two populations, ovipositing and emerging in alternate spring-tide periods, between which a considerable degree of isolation exists.

Also breeding in the mangrove swamps is a third species of the group which can be separated morphologically from the two forms referred to above by virtue of its marked melanism. This form, which is certainly a taxonomically separate species, is considered to be very much more closely related to salt-water *C. austeni* than either is to the soil breeding form. Like salt-water *C. austeni* it is autogenous in the first ovarian cycle, mates without swarming and also comprises two alternating populations. The two salt-water forms breed in different parts of the mangrove swamp, salt-water *C. austeni* in the areas dominated by *Avicennia nitida* and the related species in parts of the zones characterized by *Rhizophora racemosa* and *R. mangle*. The peaks of emergence exhibited by these two species are usually out of phase with each other by several days, presumably in association with the different soil-levels and times of inundation of their respective habitats.

For the taxonomy of this material I am indebted to Dr. W. W. Wirth of the United States National Museum, Washington, D.C. The advice is gratefully acknowledged of Dr. I. A. McGregor, director of the Medical Research Council Laboratories in Gambia, with whose permission these results are published.

D. H. Murphy

Medical Research Council Laboratories,
Fajara, near Bathurst,
Gambia.

[1] Carter, H. F., Ingram, A., and McFie, J. W. S., *Ann. Trop. Med. Parasit.*, **14**, 211 (1920).
[2] Hopkins, C. A., *Ann. Trop. Med. Parasit.*, **46**, 165 (1952).
[3] Nicholas, W. L., *Ann. Trop. Med. Parasit.*, **47**, 187 (1953).
[4] Nicholas, W. L., Kershaw, W. E., and Duke, B. O. L., *Ann. Trop. Med. Parasit.*, **49**, 455 (1955).

Sorghum Midge, *Contarinia sorghicola* (Coq.), in West Africa

Barnes[1] listed the dates of the first definite records of the sorghum midge, *Contarinia sorghicola* (Coq.), in various African countries as Sudan (1930), Nyasaland (1950), Uganda (1951), Aden Protectorate, Gambia, Kenya and Tanganyika (1952), Nigeria (1953). He suggested that these records probably reflect the fact that only in recent years have economic entomologists in Africa been able to turn their attention from cash crops to food crops, and that the midge was well established in these territories before it was first reported.

In 1958 Matthee and Oberholzer[2] reported the first appearance of the midge in South Africa and supported the alternative explanation of these records, namely, that the midge was introduced into Africa from America and has gradually spread southwards from the Sudan.

In an attempt to determine whether or not the midge was introduced to, and is spreading through, the African continent, we have begun an investigation of herbarium material of the genus *Sorghum* collected in Africa during the past century. Herbarium specimens are examined for symptoms of midge damage, and, where this is suspected, spikelets are removed for dissection under the microscope. Cecidomyiid larvæ and pupæ have been dissected from material collected more than thirty years ago, and, although larvæ and young pupæ cannot be identified beyond their genus, specific determinations can be made on the adult characters which are displayed by fully developed pupæ.

A preliminary survey of the West African section of the genus *Sorghum* in the Kew Herbarium has revealed a wealth of material from which the following new records of *C. sorghicola* have been made (Table 1).

Table 1. New Records of *Contarinia sorghicola* (Coq.) in West Africa

Country	Locality	Host	Date of collection of host
Northern Nigeria	Zaria	*S. guineense*	1929
	Munchi	*S. guineense*	1929
	Sokoto	*S. caudatum*	1929
Gold Coast	Northern Territories	*S. guineense*	1931
	—	*S. guineense*	1930

In addition, larvæ of the genus *Contarinia*, which are almost certainly *C. sorghicola*, were obtained from *S. margaritiferum* collected in Sierra Leone (Port Lokko) in 1929.

The oldest *Sorghum* collections, made in 1859, yielded no specimens of midge, possibly because these

collections are less representative, and in poorer condition than the more extensive collections made in the 1920's and 1930's. It is therefore impossible, at this stage, to determine whether the midge has or has not been introduced to Africa, since the new records presented here will easily fit into a pattern of initial introduction to West Africa, followed by distribution eastwards to the Sudan and then southwards to South Africa.

However, it is obvious that Barnes was correct in suggesting that the sorghum midge might have been overlooked by economic entomologists in Africa.

K. M. Harris *

Department of Agricultural Research,
Moor Plantation,
Ibadan, Nigeria.

* Present address: Regional Research Station, Ministry of Agriculture, Samaru, Zaria, Northern Nigeria.

[1] Barnes, H. F., *Gall Midges of Economic Importance*, **7**, 261 (Crosby Lockwood, London, 1956).
[2] Matthee, J. J., and Oberholzer, J. J., *Farming in S. Africa*, **34**, 17 (1958).

Effect of Age and Pregnancy on the Tolerance of Tsetse Flies to Insecticides

It has been found that, in the laboratory, old female *Glossina morsitans* Westw. show a considerably greater tolerance to some chlorinated hydrocarbon insecticides than do the young flies of both sexes, and in particular that pregnant flies show an exceptional degree of tolerance. Complete log-dosage/probit-mortality regression lines have been determined for dieldrin (Table 1).

Dieldrin was applied in solution in lighting kerosene as a constant volume (0·022 μl.) to the mesothorax of flies which had emerged from wild pupæ and had been given a blood feed the previous day. Pregnant flies were defined somewhat arbitrarily as those more than 50 mgm. in weight at the time of testing, and fertilized flies were those less than 50 mgm. in weight and more than 21 days old. They were a mixture of those insects which were in an early state of pregnancy or had recently larviposited or aborted. All lines were parallel, and all differences given in Table 1 are significant at $P = 0{\cdot}05$ (ref. 1). Old females also show significantly increased tolerance to DDT and γ-BHC, but males do not develop tolerance as they age. These tests have been extended to *G. swynnertoni* Aust. caught as wild adults, and pregnant females were found to be nine times as tolerant as males to DDT.

That breeding females are up to nine times as tolerant as young flies is of obvious practical significance. In a recent paper, Yeo and Simpson[3] have given a theoretical forecast of the final results to be expected from repeated applications of a non-residual insecticide to wild tsetse populations, basing their calculations on a constant kill attained at each application. The kill is usually assessed from catches of males, and for the first one or two applications, of females. They discuss the results of a number of experiments in aerial control. The apparent kill for both sexes is normally more than 90 per cent, that for males being the higher, but the final reduction at the end of the experiment is frequently consistent with a kill of about 60 per cent[2]. It is suggested that this is because the major part of the measured mortality falls on young flies, the old breeding females escaping. These form a small proportion of catches because the mean life of wild females is only 1–2 months[3] and because they come most readily to man when young[4]. Thus a high kill may be recorded without any great reduction in the breeding population. This will, of course, fall as the old flies die from natural hazards, but the result will be to delay the expected reduction in breeding and increase the value of the later applications above that assigned by Yeo and Simpson's analysis. It is also necessary to re-examine the conclusion that an inter-treatment interval close to the pupal period is the best. At the appropriate season in Central Tanganyika, the mean temperature is about 24° C.[5], the pupal period about 31 days and the mean age at deposition of the first larva 23 days[6]. Thus by the end of the third week of life the female fly is pregnant and tolerant of insecticide. This means that, with an inter-application interval of 4 weeks[2], all females emerging in the week after an application are potentially tolerant of the next.

A second consideration concerns tests for insecticidal resistance. For these, care must be taken to test only males or females of known age.

G. F. Burnett

Tropical Pesticides Research Institute,
Arusha, Tanganyika.

[1] Litchfield, J. T., and Wilcoxon, F., *J. Pharm.*, **96**, 99 (1949).
[2] Yeo, D., and Simpson, H. R., *Bull. Ent. Res.*, **51**, 631 ((1960).
[3] Jackson, C. H. N., *Biol. Rev.*, **24**, 174 (1949).
[4] Jackson, C. H. N., *Bull. Ent. Res.*, **37**, 291 (1946).
[5] Jackson, C. H. N., *Ann. Eugen., Lond.*, **10**, 332 (1941).
[6] *Notes for Field Studies of Tsetse Flies in East Africa*, E.A. Tsetse and Trypt. Res. Org. (1955).
[7] Fisher, R. A., and Yates, F., *Statistical Tables* (Edinburgh, 1948).
[8] Burnett, G. F., *Bull. Ent. Res.* (in the press).

Table 1. Toxicity Data for *G. morsitans* to which Dieldrin was applied topically in Kerosene Solution. 72 hr. Mortality, corrected for Controls (ref. 7)

Sample	Mean age (days)	Mean weight (mgm.)	LD_{50} (μgm.) and 95 per cent fid. limits		Ratio and 95 per cent fid. limits	
Young males*	4	29·2 ± 3·1	0·0017	0·0013–0·0022	1	
Fertilized females	32	43·3	0·0065	0·005 –0·0085	3·8	2·6– 5·6
Pregnant females	35	56·6	0·016	0·012 –0·021	9·4	6·4–13·8

* Young females of this batch were not tested, but in other batches there was no significant sexual difference in susceptibility (ref. 8).

Increase in Distribution of *Deraeocoris olivaceus*

The study of animal introductions and their fate provides an object lesson in ecology[1]. Some, as the leafhopper *Graphocephala coccinea*, become permanent elements of the British fauna; others, as the tingid *Stephanitis rhododendri*, achieve an initial spread but later contract; still others, such as the rhopalid *Liorhyssus hyalinus*, can do no more than maintain an occasional very temporary colony in Britain. The present communication concerns a conspicuous mirid bug, *Deraeocoris olivaceus*, which it is timely to map after a space of ten years. It occurs on *Crataegus* (hawthorn), usually becomes adult during July, and is collected by beating; its biology is known in broad outline[2].

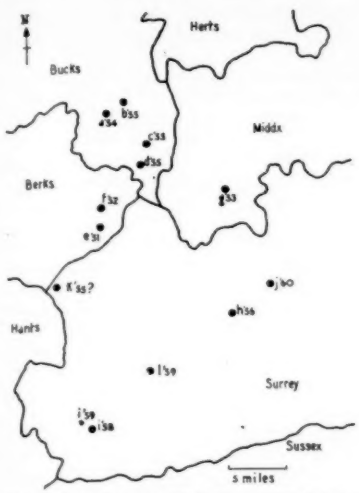


Fig. 1. Distribution of *Deraeocoris olivaceus* (Heteroptera, Miridae) in southern England, showing year of first capture at each locality. For names of localities see text

The bug was found for the first time in Britain in July 1951 at Silwood Park, Sunninghill, Berks., by Sands[3]. It is known in Europe from Greece and the Balkans to France and the Low Countries but is absent from the Baltic lands and Scandinavia. Fig. 1 shows known occurrences with the year of first capture, based on published[4] or unpublished[5] records. The localities, all in Berks., Bucks., Middx. or Surrey, are: (*a*) Burnham Beeches; (*b*) Hedgerley Park; (*c*) Langley Park; (*d*) Ditton Park; (*e*) Silwood Park; (*f*) Windsor Forest; (*g*) Hounslow Heath; (*h*) Bookham Common; (*i*) Witley and Elstead Commons; (*j*) Epsom Common; (*k*) Camberley; (*l*) Shalford. Many of these localities are well known to collectors, and it is unlikely that the bug would be overlooked for more than a year or two.

During the past few years the Herts.–Middx. and Hants–Surrey borders have been searched for the insect but so far without a capture in either Herts. or Hants. It seems, therefore, that Fig. 1 represents the present distribution, based on records during the decade 1951–60, fairly accurately. Recent checks have shown that the bug is still present at its earlier known stations. Spread of the insect from its 1951–55 area is thus shown to have been slight, save towards the south where its advance seems to be on a broadening front. The host-plant is common throughout southern England, so any restraint imposed on it is likely to be climatic. No braconid parasitism has so far been detected although its congeners in Britain are known to be attacked.

DENNIS LESTON

Green End,
Renhold, Beds.

G. E. WOODROFFE

22 Fairfield Avenue,
Datchet, Bucks.

[1] Elton, C., *The Ecology of Invasions by Animals and Man* (London, 1958).
[2] Southwood, T. R. E., and Leston, D., *Land and Water Bugs of the British Isles* (London, 1959).
[3] Sands, W. A., *Ent. Mon. Mag.*, **90**, 301 (1954).
[4] Allen, A. A., *Ent. Mon. Mag.*, **94**, 180 (1958). Woodroffe, G. E., *Ent. Mon. Mag.*, **90**, 237 (1954); *Entomologist*, **87**, 16 (1954); *Ent. Mon. Mag.*, **92**, 47, 300 (1956); *Entomologist*, **92**, 8 (1959).
[5] Allen, A. A., Leston, D., Massee, A. M., and Woodroffe, G. E. (unpublished results).

MICROBIOLOGY

New Method of preparing *Corynebacterium pyogenes* Toxoid with High Anti-hæmolysin Titre in Rabbits

Corynebacterium pyogenes is generally grown in Robertson's medium[1] when small quantities are prepared and in derivatives of this medium for vaccine production. Producing this medium for vaccines is an expensive time-consuming operation, so it was decided to investigate the possibilities of using fat-free milk as the basic medium. Strains used throughout the work were those recently isolated from bovine lesions and used at present for vaccine production.

To a 10-l. 'Pyrex' bottle was added 7 l. of fresh skimmed milk with a natural *p*H of 6·8, this being particularly important since milk with a higher acidity was found to coagulate on autoclaving. The *p*H was raised to 7·4 using 40 per cent caustic soda. To this was added 100 gm. of calcium carbonate for controlling acidity during incubation. Stoppered with cotton wool, the bottle was autoclaved at 5 lb./sq. in. for 30 min. At a higher temperature caramelization of the lactose was found to occur, resulting in a dark filtrate. After cooling to 37° C. the medium was inoculated with 10 ml. of a 48 hr. active unwashed culture of *Corynebacterium pyogenes* grown in Robertson's medium, with a hæmolysin titre of 1 : 640. A sterile rubber stopper fitted with an air filter and a length of glass tubing which almost reached to the bottom of the bottle replaced the cotton wool. During the 48-hr. incubation period at 37° C. sterile air was passed slowly through the medium; carbon dioxide was also tried without equal success, as a cloudy filtrate resulted. After 24 hr. coagulation and digestion of the casein had begun, and after 48 hr. all that remained was a small flocculer precipitate and pale-yellow supernatant, colourless in small quantities. The resultant supernatant was clarified afterwards through kieselguhr and sterile-filtered through Berkfield candles; there was very little loss of fluid during filtering. The hæmolysin titre obtained by this method was 1 : 1,000. The filtrate was toxoided by the addition of 0·5 per cent formalin and this was incubated at 37° C. over a period of 2 days. 0·5 ml. was injected intravenously into two mice which showed no toxic reaction after 24 hr. The *p*H was then altered from 5·8 to 6·8 using 40 per cent caustic soda, and 'Thiomersalate' (British Drug Houses) in the final dilution of 1 : 10,000 was used as preservative. 'Thiomersalate' was used in preference to phenol or tricresol as the latter preservatives tended to precipitate the proteins, causing an opacity in the toxoid which increased in density with time and high storage temperatures.

Rabbits used in this work were tested for natural immunity before inoculation and those possessing antibodies were not used. Their sera were titrated against 5 and 10 minimal hæmolytic dose of *Corynebacterium pyogenes* toxin. Ten rabbits were injected with 10 ml. of the toxoid intramuscularly with another injection of 15 ml. ten days later and 20 ml. 10 days after that. The animals developed no signs of shock after injection nor was any rise in temperature noted, thus proving that milk pyrogens had been destroyed. The animals were bled 14 days after the last injection. On titrating the rabbit sera against 10 minimal hæmolytic dose of *Corynebacterium pyogenes* toxin 0·00156 ml. of sera inhibited hæmolytic activity; controls were set up. This figure represents thirty-two

times the potency standard for *Corynebacterium pyogenes* set down by the British Veterinary Codex. After 2 months of retesting, the sera showed no appreciable drop in titre. I feel this method could be extended to other aerobic and anaerobic proteolytic organisms used for production of vaccine.

VALERIE LEVY

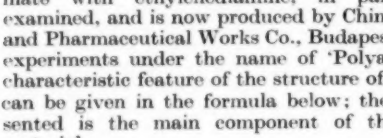
Bayer Biological Institute,
Exning,
Newmarket,
Suffolk.

[1] Robertson, J., *J. Path. and Bact.*, **20**, 327 (1916).

Antibacterial Synthetic Polypeptide Derivatives

As already stated[1], some synthetic polypeptide derivatives exhibit considerable antitubercular activity. These antibacterial polypeptide derivatives were prepared from poly-α-aminodicarboxylic-acid-esters (for example, polymethyl-α-poly-L-glutamate, polymethyl-α-poly-DL-aminoadipate) and from poly-aminodicarboxylic-acid-anhydrides (for example, anhydro-poly-aspartic-acid) with diamines (ethylenediamine, β-dialkylethylenediamines)[2].

The hydrochloride of the polypeptide derivative which was prepared from polymethyl-α-poly-L-glutamate with ethylenediamine, in particular, was examined, and is now produced by Chinoin Chemical and Pharmaceutical Works Co., Budapest, for clinical experiments under the name of 'Polyanthin'. The characteristic feature of the structure of 'Polyanthin' can be given in the formula below; the unit represented is the main component of the polymeric material:



The microbiological activity of 'Polyanthin' against different micro-organisms was further examined. The examinations, the results of which are summarized in Table 1, were performed as follows: the strains given in Table 1, were inoculated in bouillon media and incubated at 37° C. for 24 hr. With 0·1 ml. quantities of these cultures, bouillon media containing various amounts of 'Polyanthin' (see first column of Table 1) were infected. The controls were infected media with no 'Polyanthin' and non-infected culture media with 'Polyanthin'. The degree of growth of the micro-organisms characterized by crosses in Table 1 was visually evaluated after incubation at 37° C. for 24 hr.

As seen in Table 1, 5–20 γ/ml. of 'Polyanthin' restrains the growth of *Staphylococcus aureus haemolyticus* (Wood), and also of the penicillin- and sulphamide-resistant variations of *Staphylococcus aureus haemolyticus* isolated from diseased persons; of *Bacillus subtilis*, *Mycobacterium friburgensis* and *Mycobacterium smegmatis*. There was no effect against bacteria belonging to the Gram-negative group, like *Proteus vulgaris* and *Escherichia coli*, even in concentrations as high as 300 γ/ml. 'Polyanthin' seems to be active above all against those strains to which glutamine is necessary for the initiation of growth[3].

After the favourable results of the preliminary investigations clinical tests of 'Polyanthin' were also carried out. 'Polyanthin' was used, so far only for local treatments, particularly in obstetrics and gynæcology, as well as prophylactically and in cases of developed suppurations. It was used in obstetrics for the treatment of episiotomies and injuries of the vagina and cervix; in gynæcology after different operations of the vagina and abdomen in cases where the healing of the wound was disturbed.

A solution, containing 1·3 gm. 'Polyanthin' in a litre of water, was used for the different treatments either in the form of rinsings or for the soaking of gauze strips applied as tampons and covers. The controls are similar cases as those treated later with 'Polyanthin', but taken from the time before the testing of 'Polyanthin' had begun. These control cases were treated with wound powders ('Ultraseptyl-urea' (Chinoin Chemical and Pharmaceutical Works Co., Budapest), 'Dermaphorin' (United Works of Pharmaceutical and Dietetic Products, Budapest)) and wound ointments containing cod-liver oil.

From Table 2 it is seen that when 'Polyanthin' is used for the treatments the primary healing of

Table 1

Polyanthin (γ/ml.)	*Staphylococcus* (Wood)	*Staphylococcus*, penicillin-resistant	*Staphylococcus*, sulphamide-resistant	*Bacillus subtilis*	*Proteus vulgaris*	*E. coli*	*Mycobacterium friburgensis*	*Mycobacterium smegmatis*
5	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
10	—	+ + + +	+ +	—	+ + + +	+ + + +	+ + + +	+ + + +
20	—	—	—	—	+ + + +	+ + + +	—	—
50	—	—	—	—	+ + + +	+ + + +	—	—
100	—	—	—	—	+ + + +	+ + + +	—	—
300	—	—	—	—	+ + + +	+ + + +	—	—

Symbols: —, no growth; + +, partial growth; + + + +, full growth

Table 2

	Cases treated with 'Polyanthin'				No. of cases	Control cases Healing	
	Treatments		Healing				
	Prophylactin	In case of suppuration	P/I*	P/II†		P/I*	P/II†
Obstetrics	155	81	231 (97·9%)	5 (2·1%)	236	161 (68·2%)	75 (31·8%)
Gynæcology	63	65	124 (96·9%)	4 (3·1%)	128	81 (63·2%)	47 (36·8%)
Total	218	146	355 (97·5%)	9 (2·5%)	364	242 (66·5%)	122 (33·5%)

* P/I, healed per primam. † P/II, healed per secundam

wounds is 97·5 per cent, while without 'Polyanthin' the primary healing of wounds is only about 66·5 per cent.

Further investigations for the extension of the clinical applicability of 'Polyanthin' (for example, tuberculosis, surgery, general medicine) are in progress.

K. Kovács
A. Kótai

Institute of Organic Chemistry, University, Budapest, Muzeum krt. 4/b.

I. Szabó

National Institute for Tuberculosis, Korányi, Budapest 124.

R. Mecseki

Margaret-Hospital, Budapest III.

[1] Kovács, K., Kótai, A., and Szabó, I., *Nature*, **185**, 266 (1960).
[2] Kovács, K., and Kótai, A., *Acta Chim. Hung.*, **21**, 453 (1959). Kótai, A., Kovács, K., and Császár, J., *ibid.*, **21**, 461 (1959).
[3] Anderson, C. G., *Intro. to Bact. Chem.*, 106 (Williams and Wilkins Co., Baltimore, 1948).

Nature of Colicine Receptors

Colicines are unique among antibiotics in being specifically adsorbed on to the surface of bacteria in a manner analogous to antibodies and bacteriophages. Besides being removed from solution by adsorption on sensitive bacteria[1], they can be inactivated by extracts of sensitive, but not of resistant, organisms[2]. The nature of colicine receptors on the bacterial surface has not so far been elucidated. This communication describes some preliminary observations on the subject.

Absorption experiments were undertaken, using two of Fredericq's strains, namely *Shigella sonne P9*, producing colicine *S3*, and the colicine-sensitive *Escherichia coli C6*. When colicine was mixed with preparations of the sensitive *E. coli*, each killed by a different procedure, then centrifuged and the residual colicine assayed, a number of properties of the receptor sites were demonstrated. They were unaffected when the organisms were killed by heat at 65° C. for 30 min., by exposure to 0·3 per cent formalin or 2 per cent phenol, but were destroyed by heating at 100° C. for 1 hr. or exposure to 80 per cent ethanol.

The residual colicine was titrated by the agar diffusion technique developed by E. van Horn (personal communication). By this technique small differences in colicine titre can be detected, and results may also be obtained with a more than hundred-fold dilution of the original colicine. Partial absorption of colicine could thus be detected but, in fact, in the absorption experiments described here either all or none of the colicine was removed from the solution.

The results obtained with the absorption experiments were corroborated by assaying the colicine on agar plates inoculated with a mixture of viable and killed organisms. The inoculum consisted of 5–10 × 10^6/ml. viable organisms of the sensitive *E. coli*, mixed with a suspension of 5–10 × 10^8/ml. organisms of the same *E. coli* killed by the methods used in the absorption experiments. The killed suspensions in which the receptor site had been destroyed did not materially alter the size of the inhibition zones; those in which the receptor sites were intact adsorbed colicine and hence the inhibition zones were significantly reduced in size. The amount of reduction was comparable with that obtained by an inoculum of viable cells, substituted for the killed organisms[3].

From these observations it appears that the receptor, at least for this particular colicine, resembles most closely a flagellar or, to a lesser extent, a fimbrial antigen. *E. coli C6* is non-motile, which lends particular interest to the finding of a receptor substance on its surface having the properties of a flagellar antigen.

Anna Mayr-Harting

Department of Bacteriology, University of Bristol.

[1] Jacob, F., Siminovitch, L., and Wollman, E., *Ann. Inst. Pasteur*, **83**, 295 (1952).
[2] Bordet, P., and Beumer, J., *C.R. Soc. Biol.*, **142**, 259 (1948).
[3] van Horn, Elizabeth A., M.Sc. thesis, University of Bristol (1961).

MISCELLANEOUS

A Simple Flattener for holding Filter Papers on Planchettes for Radioactive Counting

The use of a 'Millipore' chimney and filter paper provides a convenient means of collecting radioactive material for measurement of its activity. A major disadvantage of this system arises from the curling of these papers by the precipitated materials and the allied difficulties of fitting them in the planchette. The use of doubly gummed 'Scotch' tape provides only a partial solution to this problem, and if the material on the filter paper is to be analysed chemically afterwards, the tape adhesive may interfere. The use of liquid adhesives and, in the case of 'Millipore' filters, of solvents also does not solve this problem. The amount of collected material is limited by the point at which the precipitate will flake and not hold to the paper. Recently we devised a simple spring mechanism to hold the paper in the carriers.

The precipitates are collected on the surface of hardened small-pore filter paper (for example, Whatman No. 42) or modified cellulose papers ('Millipore' filters) and dried in a desiccator. The flatness of the resulting material is a function of the amount of precipitated material collected. The diameters of the papers are slightly smaller than the inside diameter of the planchettes used (Nuclear-Chicago sample pan 1¼-in. in diameter). The papers are fixed inside the planchette by use of a 330° arc of stainless steel spring wire No. 24 gauge, so made that the arc has a diameter ¼ in. greater than the planchette (Fig. 1). The wire is placed within the planchette and on the paper by compressing it with the thumb and forefinger and placing the closed end against the wall of the planchette. The spring is then permitted to expand slowly below the level of the planchette side wall, and final adjustment is made with a pair of forceps.

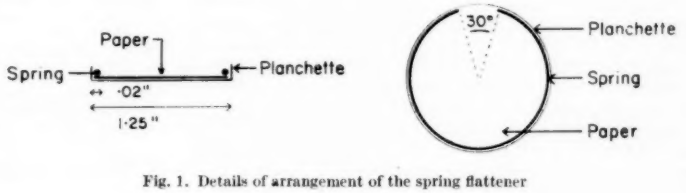


Fig. 1. Details of arrangement of the spring flattener

We have found these uniquely successful in holding the papers and their contents flat; they are simple to use and easy to clean. They will withstand brief washing in dichromate cleaning solution, may be autoclaved, and can be made by most machine shops. A convenient material, though not stainless steel, is commercially available piano wire. Thicker wire will provide additional strength where required.

This work was supported in part by U.S. Public Health Service grants *H*–3174 and *E*–3854.

Edward A. Smuckler
Earl P. Benditt

Department of Pathology,
University of Washington,
Seattle 5, Washington.

Chemico-Linguistics: Computer Translation of Chemical Nomenclature

I wish to report what is believed to be the first successful mechanical translation of chemical names into chemical formulæ. Tsukermann and Terentiev[1] have indicated their intention to work on this problem.

Programming the initial experiments was simplified by imposing several restrictions. Simulated tests by manual methods have been successfully performed without these restrictions: (*a*) longest carbon chains allowed are ten carbon atoms in length; (*b*) maximum length of chemical names is 59 characters; (*c*) elements other than carbon, hydrogen, oxygen, nitrogen, sulphur, bromine, chlorine, fluorine and iodine were eliminated.

The most significant aspect of this research is the linguistic study of chemical nomenclature which preceded, for several years, the development of the computer programme[2].

In effect, a recognition grammar for organic chemistry has been written which can be used not only for mechanical indexing but also for teaching chemical nomenclature. Using Harris's methods[3,4] for grammatical analyses both the morphology and syntax of chemical nomenclature were analysed. Morphemes, allomorphs, homonyms, etc., were identified as well as their roles in the generation of molecular formulæ.

The difficult problem of calculating hydrogen values was overcome by application of the following formula, which is a modification of Soffer's[5] formula for determining double bonds and cyclic structures:

$$H = 2(\Sigma M_C - \Sigma M_{db} + 1) + \Sigma M_N - \Sigma M_X$$

where M_i is an expression indicating the summation of the computational values represented by morpheme class i. For example, M_C is the number of carbon atoms implied by all morphemes in the chemical names which contain carbon. This completely resolved the tedious, though solvable, problem of constructing more complex rules for hydrogen calculation which are required when one attempts to follow the 'natural' inclination to record methyl as CH_3, amino as NH_2, etc.

It is the ultimate objective of this work to generate accurate molecular formulæ for all chemical names and also to display and print ideographs, that is, structural diagrams. Once the initial linguistic problems have been completely resolved, this can be done. It has already been shown that computers can be instructed to draw such diagrams[6,7]. However, neither Opler nor Waldo began with uncoded chemical names. It is one thing to programme a computer to draw a diagram for a known specific chemical. In this case, a human coder works from a diagram drawn by someone and calculates a cipher that is used by the computer to draw the same diagram. It is another matter to translate, without human intervention, from chemical names to structural diagrams.

The most difficult problem one encounters when trying to include all methods of naming chemicals, rather than just systematic International Union of Pure and Applied Chemistry names, is the ambiguity of different ring-numbering systems. While this does not affect the ability to calculate molecular formulæ, it does affect the ease of creating entirely accurate diagrams. In such cases the computer will render an invaluable service in reminding the chemist that several alternatives must be considered. It may also remind authors to stipulate whether they are following International Union of Pure and Applied Chemistry or some other system.

From a linguistic point of view, it is an interesting observation that the basic language of all naming systems in organic chemistry is essentially the same. While two chemists will name the same compound differently, both will be able to draw the same structural diagram. In this sense, the use of different systems corresponds to the problem of handling dialects rather than treatment of separate distinct languages. In addition, the complete understanding of the mechanisms for translating the language of organic chemistry provides useful experimental data for the more general problem of mechanical translation. If we cannot solve the problem of translating chemical nomenclature, there would seem to be little hope for translating natural languages such as English with a machine.

The following chemical compounds (Table 1) illustrate the capabilities of the present computer programme. While a *Univac* I was used for this experiment, the programme can be modified without too much difficulty for smaller computers such as the I.B.M. 650 (ref. 8).

Table 1

Chemical name	Molecular formula
1,4-*bis*[*bis*(3-Diethylaminopropyl)amino]butane	$C_{32}H_{72}N_6$
2-[(2-Aminopropyl)ethylamino]ethanol	$C_7H_{18}N_2O$
8-Hydroxy-6-octene-2,4-diynenitrile	C_8H_5NO
1,3,3,4,4-Pentachloro-2-methyl*cyclo*butene	$C_5H_3Cl_5$
2-Phenyl-2,4,6-*cyclo*heptatriene-1-one	$C_{13}H_{10}O$
7-(2,4,5-Trichlorophenoxy)heptanoic acid	$C_{13}H_{15}Cl_3O_3$
Ethyl 2-cyano-5-phenyl-2,4-pentadienoate	$C_{14}H_{13}NO_2$
2-Naphthyl-2-*cyclo*hexen-1-one	$C_{16}H_{14}O$
Diphenyl-3-butynol	$C_{16}H_{14}O$

I wish to thank Dr. J. O'Connor and Mr. C. Meunch for their help in writing this programme, Dr. C. Borkowski for fruitful discussions on problems of mechanical translation, and Prof. N. Rubin, who acted as my chemical 'informant'.

E. Garfield

Institute for Scientific Information,
33 South 17th St.,
Philadelphia 3.

[1] Tsukermann, A. M., and Terentiev, A. P., *Proc. Intern. Conf. on Standards for a Common Language for Machine Searching and Translation*, **1**, 493 (Interscience Press, 1960).
[2] Garfield, E., doctoral dissertation, Univ. of Pennsylvania (1961).
[3] Harris, Z. S., *Methods in Structural Linguistics* (Univ. of Chicago Press, 1951).
[4] Harris, Z. S., *Annual Report of the Computing Centre* (Univ. of Pennsylvania, Philadelphia, 1960).
[5] Soffer, M. D., *Science*, **127**, 880 (1958).
[6] Waldo, W. H., and DeBacker, M., *Proc. Intern. Conf. Sci. Information*, 711 (Washington, 1958).
[7] Opler, A., and Baird, N., 133rd Nat. Meeting of the Amer. Chem. Soc., April 1958; *Amer. Documentation*, **10**, 59 (1958).
[8] Garfield, E., *An Algorithm for Translating Chemical Names to Molecular Formulas* (Inst. Scientific Information, 1961).

FORTHCOMING EVENTS

(Meetings marked with an asterisk • are open to the public)

Monday, October 16

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. E. Sherlock: "Advances in the Chemistry of Phosphorous Insecticides".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons"•. (First of seven lectures in War Studies. Further lectures on October 23, 30, November 6, 13, 20 and 27.)

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. A. Rodger: "Occupational Versatility and Planned Procrastination".• (Inaugural Lecture.)

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. S. G. Clarke: "Chromate Passivation of Brass".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.45 p.m.—Mr. C. F. Price: "Experimental Techniques Developed for Determining the Performance of Fuel Elements in Nuclear Reactors" (Chairman's Address).

Monday, October 16—Wednesday, October 18

INSTITUTE of METALS (in the Assembly Hall of the Royal Commonwealth Society, Craven Street, London, W.C.2), at 9.30 a.m. on Monday and 9 a.m. on Tuesday and Wednesday—International Conference on "The Metallurgy of Beryllium".

Tuesday, October 17

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. P. J. Newbould: "Nature Reserves in Britain: Can We Afford Them?"•

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Place of Digital Computers in the Teaching of Electrical Engineers" opened by Dr. P. D. Aylett.

Wednesday, October 18

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. S. Forrest: "Research and Transmission" (Chairman's Address).

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. J. W. Nichols and Mr. A. C. MacKellar: "Some Recent Developments in Marine Navigational Aids".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Meeting on "Volatile Corrosion Inhibitors". Mr. E. Ll. Evans: "Principles"; Mr. R. I. Barraclough: "Practical Applications".

INSTITUTE OF INFORMATION SCIENTISTS (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Sir Lindor Brown, F.R.S.: Presidential Address.

INSTITUTE OF WOOD SCIENCE (at Carpenters Hall, Throgmorton Avenue, London, E.C.2), at 7 p.m.—Mr. P. O. Reece: "The Establishment of Safety Factors and Stresses for Timber".

OIL AND COLOUR CHEMISTS' ASSOCIATION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. L. M. Baraken: "Solvents in the Surface Coatings Industry".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Miss Maureen Wilson: "The Use of Bacteriophages in Epidemiology".

Thursday, October 19

UNIVERSITY COLLEGE, LONDON (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. M. Mary Douglas: "Cult and Ordeal as Techniques of Sorcery Control in the Belgian Congo".•

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Discussion of a paper by Mr. J. T. M. Taylor on "Mining Practice on the Kolar Gold Field, India".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific papers by Dr. Anna B. Hastings and Dr. H. G. Vevers.

UNIVERSITY OF LONDON (at Westfield College, Kidderpore Avenue, London, N.W.3), at 5.15 p.m.—Mrs. Grace Wyndham Goldie: "Television in Society".•

LONDON PLANETARIUM (in collaboration with the ROYAL SOCIETY and the ROYAL ASTRONOMICAL SOCIETY, at the London Planetarium, Marylebone Road, London, N.W.1), at 5.30 p.m.—Dr. Joseph Needham, F.R.S.: "Astronomy in Classical China".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. S. Parkes, F.R.S.: "External Factors in Mammalian Reproduction".• (Second of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

SOCIETY OF INSTRUMENT TECHNOLOGY (at the Royal Institution, Albemarle Street, London, W.1), at 6 p.m.—Sir George Thomson, F.R.S.: "The Inspiration of Science" (First Thomson Lecture).

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the SOUTH-EAST ESSEX TECHNICAL COLLEGE SCIENTIFIC SOCIETY, at the South-East Essex Technical College, Longbridge Road, Dagenham, Essex), at 7 p.m.—Dr. S. I. Levy: "Chemical Patents".

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Scientific Papers.

BRITISH INSTITUTE OF RADIOLOGY (in the Reid-Knox Hall, 32 Welbeck Street, London, W.1), at 8 p.m.—Ordinary Meeting followed by Mr. F. G. Spear: "Questioning the Answers" (Presidential Address).

UNIVERSITY OF LONDON (in Swanley Hall, Withersdane, Wye College, near Ashford, Kent), at 8.15 p.m.—Prof. J. D. Bernal, F.R.S.: "Science in History".

Thursday, October 19—Friday, October 20

INSTITUTION OF CHEMICAL ENGINEERS, GRADUATES AND STUDENTS SECTION (at the Royal Overseas League, St. James's, London, S.W.1)—Symposium on "Scale-up and Pilot Plants".

Friday, October 20

BRITISH PSYCHOLOGICAL SOCIETY, OCCUPATIONAL PSYCHOLOGY SECTION (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Dr. R. A. M. Gregson: "Operational Research, Psychological Information and Managerial Decisions".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. T. A. Brock: "Petroleum Products in Cosmetics".

Saturday, October 21

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. E. Milne-Redhead: "A Plant-Collecting Expedition to Southern Tanganyika".•

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SCIENTIFIC OFFICER (male, with a good honours degree), for genetical, physiological and breeding research with cereals—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (October 21).

MAP CURATOR/LIBRARIAN (preferably with qualifications in geography and librarianship) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Sheffield (October 23).

RESEARCH PHYSICIST, Senior Scientific Officer (with an honours degree together with several years appropriate experience) at the Defence Standards Laboratories, Finsbury, near Adelaide, South Australia, for basic studies relating to the effects of light, X- and gamma-radiation on photoconductive materials and the physics of electrophotography—The Senior Representative (AV.125/6), Department of Supply, Australia House, Strand, London, W.C.2 (October 26).

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Nottingham (October 28).

LECTURER OR DEMONSTRATOR (Assistant Lecturer) IN PHYSIOLOGY—The Registrar, The University, Leeds 2 (October 31).

LECTURER OR ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Sheffield (November 4).

JUNIOR RESEARCH FELLOW (with a good first degree in zoology) IN LOCUST PHYSIOLOGY IN THE DEPARTMENT OF ZOOLOGY, University College of Rhodesia and Nyasaland, for work on the water relations of locusts—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 8).

READER IN CHEMICAL PATHOLOGY at the Postgraduate Medical School of London—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 8).

LECTURER IN PHILOSOPHY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 15).

LECTURER (with special interest in plant physiology or morphology or taxonomy) IN THE DEPARTMENT OF BOTANY, University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (November 15).

LECTURER/SENIOR LECTURER IN ORGANIC CHEMISTRY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 17).

LECTURER (with a good honours degree and experience in research or teaching, and preferably qualifications in the history and philosophy of science) IN THE SCHOOL OF PHILOSOPHY, University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 17).

SENIOR LECTURER (with a higher degree or equivalent qualifications) and a LECTURER (with an honours degree) IN THE SCHOOL OF NUCLEAR ENGINEERING, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia November 17).

XUM

LECTURER and an ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Registrar, The University, Nottingham (November 22).

LECTURER (with an honours degree in chemistry and preferably a higher degree, and experience and a special interest in modern physical methods) IN THE DEPARTMENT OF CHEMISTRY, Newcastle University College—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 24).

CHAIR OF PHARMACOLOGY in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

KEEPER (with an appropriate university degree, and preferably museum experience and/or a knowledge of herbarium work) OF THE DEPARTMENT OF BOTANY—The Secretary, National Museum of Wales, Cardiff (November 30).

LECTURERS (2) IN PHYSIOLOGY; and a LECTURER IN PHARMACOLOGY at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

SENIOR LECTURER IN METEOROLOGY; and a LECTURER IN GLACIOLOGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (with a degree or H.N.C. in chemistry or equivalent), for work mainly concerned with the analysis of foods and feeding-stuffs of tropical origin—The Director, Tropical Products Institute (D.S.I.R.), 56 Gray's Inn Road, London, W.C.1, quoting Ref. YE.709/9.

ASSISTANT LECTURER, Grade B (with a good honours degree in mathematics and preferably some teaching or research experience), IN MATHEMATICS—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

ENTOMOLOGIST (with a good honours degree in biology, with entomology as a special subject, and not less than two years postgraduate research experience in entomology) at the West African Institute for Trypanosomiasis Research, to carry out laboratory and field investigations on tsetse flies, at Kaduna, Northern Nigeria—The Crown Agents, 4 Millbank, London, S.W.1, quoting Ref. M3B/53514/NF.

JUNIOR TECHNICAL OFFICER (with G.C.E. at "A" level in two science subjects, one of which should be botany or biology), for work in a section investigating the cytogenetic effects of radiation—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks.

LECTURER (with a good honours degree in physics and preferably a Ph.D. degree, and preferably experimental research experience in some aspects of plasma physics and an interest in high-current discharge work) IN THE DEPARTMENT OF NATURAL PHILOSOPHY—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

LIBRARIAN (with a knowledge of chemistry or physics) IN THE DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY—Prof. A. R. Ubbelohde, F.R.S., Imperial College of Science and Technology, London, S.W.7.

POSTDOCTORAL FELLOW, for research in flame spectroscopy or a related subject—Dr. A. G. Gaydon, F.R.S., Chemical Engineering Department, Imperial College, London, S.W.7.

POSTDOCTORAL FELLOW, for research in modern concepts between structure and biological activity or organic molecules, organic synthesis and stereochemistry—Prof. B. Belleau, Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada.

RESEARCH ASSISTANT (Graduate), to undertake cell counts as part of an investigation on the growth of cereal seedlings—The Secretary, Agricultural Research Council Unit of Plant Physiology, Imperial College of Science and Technology, London, S.W.7.

SCIENTIFIC OFFICER (male or female, with an honours degree in science, pharmacy or biology) IN THE BLOOD TRANSFUSION SERVICE, for duties which will consist of technical work in the Blood Transfusion Service Laboratories—The Director, Blood Transfusion Service, Roby Street, Manchester 1.

SENIOR LABORATORY TECHNICIAN, Grade 1 (with appropriate qualifications and experience)—The Head Master, King Edward VI School, Southampton.

STATISTICIANS (2) (with good qualifications in mathematics and statistics), to engage in the development of biometric techniques for medical research and the application of electronic computers to medical problems—The Secretary, Marischal College, The University, Aberdeen.

TECHNICIAN AT THE MARINE BIOLOGICAL STATION, Port Erin—The Registrar, The University, Liverpool 3, quoting Ref. CV/N/M.B.S.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Institute of Personnel Management. Job Analysis. By H. E. Roff and T. E. Watson. Pp. 40. 7*s*. 6*d*. Supervisory Training. By J. R. Armstrong. Pp. 51. 7*s*. 6*d*. (London : Institute of Personnel Management, 1961.) [148

Department of Scientific and Industrial Research : National Physical Laboratory. Notes on Applied Science No. 22 : Capacitors, Inductors and Resistors at Radio Frequencies. By Dr. T. I. Jones. Pp. iv + 24. (London : H.M. Stationery Office, 1961.) 1*s*. 9*d*. net. [148

Britain's Cultural Crisis. By M. C. Goodall. Pp. 4. (Hove, Sussex : M. C. Goodall, 16 King's Gardens, 1961.) [158

C.I.B.A. (A.R.L.), Ltd. Technical Notes, No. 224, (August, 1961) : Boat Building by Yarecraft—Colf-moulded and Hard-Chine Hulls Bonded with "Aerolite" 300. Pp. 8. (Duxford : C.I.B.A. (A.R.L.), Ltd., 1961.) [158

Royal Observatory Bulletins. No 27 : Three-colour Photometry in the Southern Hemisphere—NGC 6383, NGC 6405 and Standard Stars. By O. J. Eggen. Pp. C61–70. (London : H.M. Stationery Office, 1961.) 3*s*. net. [158

Building Research Station Digest, No. 13, (Second Series) : Concrete Mix Proportioning and Control—1. Pp. 4. (London : H.M. Stationery Office, 1961.) 4*d*. [158

Control of Public Expenditure. Pp. 33. (Cmnd. 1432). (London : H.M. Stationery Office, 1961.) 1*s*. 9*d*. net. [258

Other Countries

Mental Health Book Review Index, Vol. 6, Whole No. 11, July 1961. Compiled by the Editorial Committee and Contributing Librarians. (Sponsored by the World Federation for Mental Health; The International Council of Psychologists; The American Foundation for Mental Hygiene; and The Research Center for Mental Health, New York University.) Pp. xvii + 62. (Flushing, N.Y.: Miss Lois Afflerbach, Paul Klapper Library, Queens University, 1961.) [158

Institute for Scientific Information, Philadelphia. Descriptive Brochure. Pp. 18. (Philadelphia, Penn.: Institute for Scientific Information, 1961.) [158

Chicago Natural History Museum. Fieldiana: Anthropology, Vol. 52: Mineral Creek Site and Hooper Ranch Pueblo, Eastern Arizona. By Paul S. Martin, John B. Rinaldo and William A. Longacre. Pp. 181. (Chicago: Chicago Natural History Museum, 1961.) 5.50 dollars. [158

New South Wales: Department of Agriculture. Contributions from the New South Wales National Herbarium. Flora Series, No. 101. Part 1: Papilionaceae. By Joy Thompson. Pp. ii + 91. (Sydney: Government Printer, 1961.) [158

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 22: Plane Coordinate Intersection Tables (2½-Minute), Mississippi. Pp. iii + 92. 50 cents. Publication 65-1, Part 40: Place Coordinate Intersection Tables (2½-Minute), Tennessee. Pp. iii + 58. 40 cents. (Washington, D.C.: Government Printing Office, 1961.) [158

Arctic Institute of North America. Technical Paper No. 6: The Medusae of the Chukchi and Beaufort Seas of the Arctic Ocean including the description of a New Species of *Eucodonium* (Hydrozoa: Anthomedusae). By Cadet Hand and Lai Bing Kan. Pp. 23. (Montreal: Arctic Institute of North America, 1961.) 1 dollar. [158

Canada. Publications of the Dominion Astrophysical Observatory, Victoria, B.C. Vol. 11, No. 16: Curve of Growth of C_2 Absorption Bands Applied to the Problem of the C^{12}/C^{13} Abundance Ratio. By John L. Climenha a. Pp. 307–338. 25 cents. Vol. 117, No. 17: The Light-Ratio and Secondary Spectrum of the Eclipsing Binary Zeta Aurigae. By E. K. Lee and K. O. Wright. Pp. 339–353. Vol. 11, No. 10: Some Spectroscopic Observations of the Supergiants 67 Ophiuchi, 55 Cygni and χ^2 Orionis. By Anne B. Underhill. Pp. 353–362. (Ottawa: Queen's Printer, 1960 and 1961.) [158

University of California Publications in Geological Sciences. Vol. 40, No. 1: California Pleistocene Paleotemperatures. By James W. Valentine and Robert F. Meade. Pp. 1–46. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1961.) 1 dollar. [158

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 42, (June, 1961): Voting Population at Record High. Recent Variations in Pneumonia-Influenza Mortality. Longevity of Industrial Policyholders in 1960. Accident Death Rates Decrease in Most States. Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.) [158

Institutt for Atomenergi: Kjeller Research Establishment, Norway. Quarterly Progress Report, January–March, 1961. Pp. iv + 41 + 35 figures. (Kjeller, Lillestrøm: Institutt for Atomenergi, Kjeller Research Establishment, 1961.) [158

National Research Council of Canada: Radio and Electrical Engineering Division. NRC No. 6404: Energy Transfer in Polyacene Solid Solutions, II—a Bibliography for 1957–1960. By F. R. Lipsett. Pp. i + 65. (Ottawa: National Research Council, 1961.) 50 cents. [158

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 974: Nehmen Lufteiektrische Faktoren Einfluss auf die Aktivitätswechsel Kleiner Insekten, insbesondere auf die Häutungs und Reproductionszahlen von Blattläusen Von. Dr. Else Haine. Pp. 86. (Köln und Opladen: Westdeutscher Verlag, 1961.) 24.30 D.M. [158

United States Department of the Interior: Geological Survey. Bulletin 1032–F: Petrography and Origin of Xenotime and Monazite Concentrations, Central City District, Colorado. By E. J. Young and P. K. Sims. Pp. iii + 273–299. 20 cents. Bulletin 1070–C: Distribution of Uranium in Rocks and Minerals of Mesozoic Batholiths in Western United States. By E. S. Larsen, Jr., and David Gottfried. Pp. v + 65–103. Bulletin 1084–J L A Constant-Feed Direct-Current Arc. By C. S. Annell and A. W. Helz. Pp. iv + 231–251. 15 cents. Bulletin 1084–K: Beryllium Content of American Coals. By T. Stadnichenko, P. Zubovic and Nola B. Sheffey. Pp. iv + 295 + 2 plates. Bulletin 1102: Stratigraphy and Refractory Clayrocks of the Dakota Group Along the Northern Front Range, Colorado. By Karl M. Waagé. Pp. v + 154 + 8 plates. Bulletin 1123–A: Geology of the Dubuque South Quadrangle, Iowa-Illinois. By C. Ervin Brown and Jesse W. Whitlow. Pp. v + 93 + 7 plates. (Washington, D.C.: Government Printing Office, 1960 and 1961.) [258

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office